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in 70% ethanol for 12 hours, and embedded in paraffin. After a 30 minute treatment with 0.1% pepsin in 0.1 M HCl at room temperature to denature DNA, staining was performed using mouse monoclonal anti-BrdU antibodies (Amersham). It appeared that the VEGF-C-receptor interaction in the transgenic mice transduced a mitogenic signal, because, in contrast to littermate controls, the lymphatic endothelium of the skin from young K14-VEGF-C mice showed increased DNA synthesis as demonstrated by BrdU incorporation followed by staining with anti-BrdU antibodies. This data further confirms that VEGF-C acts as a true growth factor in mammalian tissues.

In related experiments, a similar VEGF transgene did not induce lymphatic proliferation, but caused enhanced density of hyperpermeable, tortuous blood microvessels instead.

Angiogenesis is a multistep process which includes endothelial proliferation, sprouting, and migration. See Folkman *et al.*, *J. Biol. Chem.*, 267: 10931-10934 (1992). To estimate the contribution of such processes to the transgenic phenotype, the morphology and function of the lymphatic vessels was analysed using fluorescent microlymphography using techniques known in the art. See Leu *et al.*, *Am. J. Physiol.*, 267: 1507-1513 (1994); and Swartz *et al.*, *Am. J. Physiol.*, 270: 324-329 (1996). Briefly, eight-week old mice were anesthetized and placed on a heating pad to maintain a 37°C temperature. A 30-gauge needle, connected to a catheter filled with a solution of FITC-dextran 2M (8 mg/ml in PBS), was injected intradermally into the tip of the tail. The solution was infused with a constant pressure of 50 cm water (averaging roughly 0.01 microliters per minute flow rate) until the extent of network filling remained constant (approximately 2 hours). Flow rate and fluorescence intensity were monitored continuously throughout the experiment. In these experiments, a typical honeycomb-like network with similar mesh sizes was observed in both control and transgenic mice, but the diameter of lymphatic vessels was about twice as large in the transgenic mice, as summarized in the table below. (The intravital fluorescence microscopy of blood vessels

was performed as has been described in the art. See Fukumura *et al.*, *Cancer Res.*, 55: 4824-4829 (1995).)

Structural parameters of lymphatic and blood vessel networks				
		transgenic	control	P-value**
		(n=4)	(n=5)	
5 lymphatic vessels*	diameter	142.3±26.2	68.2±21.7	.0143
	horizontal mesh size***	1003±87.1	960.8±93.1	.2207
	Vertical mesh size	507.3±58.9	488.8±59.9	.5403
		(n=3)	(n=6)	
blood vessels	median diameter	8.3±0.6	7.6±1.1	.1213
	vessel density, cm/cm ²	199.2±6.6	216.4±20.0	.3017

n=number of animals

* mean (μm)±SD

10 **Mann-Whitney test

***mesh size describes vessel density

Some dysfunction of the abnormal vessels was indicated by the fact that it took longer for the dextran to completely fill the abnormal vessels. Injection of FITC-dextran into the tail vein, followed by fluorescence microscopy of the ear, showed that the blood vascular morphology was unaltered and leukocyte rolling and adherence appeared normal in the transgenic mice. These results suggest that the endothelial proliferation induced by VEGF-C leads to hyperplasia of the superficial lymphatic network but does not induce the sprouting of new vessels.

20 These effects of VEGF-C overexpression are unexpectedly specific, especially since, as described in other examples, VEGF-C is also capable of binding to and activating VEGFR-2, which is the major mitogenic receptor of blood vessel endothelial cells. In culture, high concentrations of VEGF-C stimulate the growth and migration of bovine capillary endothelial cells which express VEGFR-2, but not significant amounts of VEGFR-3. In addition, VEGF-C induces vascular permeability in the Miles assay [Miles,

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A. A., and Miles, E. M., *J. Physiol.*, 118:228-257 (1952); and Uchida, *et al.*, *Proc. Soc. Exp. Biol. Med.*, 133:1384-1387 (1970)], presumably via its effect on VEGFR-2. VEGF-C is less potent than VEGF in the Miles assay, 4- to 5-fold higher concentrations of VEGF-C $\Delta\Delta$ CHis being required to induce the same degree of permeability. *In vivo*, the specific effects of VEGF-C on lymphatic endothelial cells may reflect a requirement for the formation of VEGFR-3xVEGFR-2 heterodimers for endothelial cell proliferation at physiological concentrations of the growth factor. Such possible heterodimers may help to explain how three homologous VEGFs exert partially redundant, yet strikingly specific biological effects.

10 The foregoing *in vivo* data indicates utilities for both (i) VEGF-C polypeptides and polypeptide variants and analogs having VEGF-C biological activity, and (ii) anti-VEGF-C antibodies and VEGF-C antagonists that inhibit VEGF-C activity (*e.g.*, by binding VEGF-C or interfering with VEGF-C/receptor interactions. For example, the data indicates a therapeutic utility for VEGF-C polypeptides in patients wherein growth of lymphatic tissue may be desirable (*e.g.*, in patients following breast cancer or other surgery where lymphatic tissue has been removed and where lymphatic drainage has therefore been compromised, resulting in swelling; or in patients suffering from elephantiasis). The data indicates a therapeutic utility for anti-VEGF-C antibody substances and VEGF-C antagonists for conditions wherein growth-inhibition of lymphatic tissue may be desirable
15 (e.g., treatment of lymphangiomas). Accordingly, methods of administering VEGF-C and VEGF-C variants, analogs, and antagonists are contemplated as methods and materials of the invention.

Example 30

Expression of VEGF-C and Flt4 in the Developing Mouse

25 Embryos from a 16-day post-coitus pregnant mouse were prepared and fixed in 4% paraformaldehyde (PFA), embedded in paraffin, and sectioned at 6 μ m. The sections were placed on silanated microscope slides and treated with xylene, rehydrated, fixed for 20 minutes in 4% PFA, treated with proteinase K (7mg/ml; Merck, Darmstadt, Germany) for 5 minutes at room temperature, again fixed in 4% PFA and treated with
30 acetic anhydride, dehydrated in solutions with increasing ethanol concentrations, dried and used for *in situ* hybridization.

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In situ hybridization of sections was performed as described (Västrik *et al.*, *J. Cell Biol.*, 128:1197-1208 (1995)). A mouse VEGF-C antisense RNA probe was generated from linearized pBluescript II SK+ plasmid (Stratagene Inc.), containing a fragment corresponding to nucleotides 499-979 of mouse VEGF-C cDNA, where the 5 noncoding region and the BR3P repeat were removed by Exonuclease III treatment. The fragment had been cloned into the *EcoRI* and *HindIII* sites of pBluescript II SK+. Radiolabeled RNA was synthesized using T7 RNA Polymerase and [³⁵S]-UTP (Amersham, Little Chalfont, UK). About two million cpm of the VEGF-C probe was applied per slide. After an overnight hybridization, the slides were washed first in 2x SSC and 20-30 mM DDT for 1 hour at 50°C. Treatment continued with a high stringency wash, 4x SSC and 20 mM DTT and 50% deionized formamide for 30 minutes at 65°C followed by RNase A treatment (20 µg/ml) for 30 minutes at 37°C. The high stringency wash was repeated for 45 minutes. Finally, the slides were dehydrated and dried for 30 minutes at room temperature. The slides were dipped into photography emulsion and exposed for 4 weeks. Slides were developed using Kodak D-16 developer, counterstained with hematoxylin and mounted with Permount (FisherChemical).

For *in situ* hybridizations of Flt4 sequences, a mouse Flt4 cDNA fragment covering bp 1-192 of the published sequence (Finnerty *et al.*, *Oncogene*, 8:2293-2298 (1993)) was used, and the above-described protocol was followed, with the following exceptions. Approximately one million cpm of the Flt4 probe were applied to each slide. The stringent washes following hybridization were performed in 1x SSC and 30 mM DTT for 105 minutes.

Darkfield and lightfield photomicrographs from these experiments are presented in commonly-owned PCT patent application PCT/FI96/00427, filed August 01, 1996, incorporated by reference herein. Observations from the photomicrographs are summarized below.

The most prominently Flt4-hybridizing structures appeared to correspond to the developing lymphatic and venous endothelium. A plexus-like endothelial vascular structure surrounding the developing nasopharyngeal mucous membrane was observed. The most prominent signal using the VEGF-C probe was obtained from the posterior part of the developing nasal conchae, which in higher magnification showed the epithelium surrounding loose connective tissue/forming cartilage. This structure gave a strong *in situ*

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hybridization signal for VEGF-C. With the VEGF-C probe, more weakly hybridizing areas were observed around the snout, although this signal is much more homogeneous in appearance. Thus, the expression of VEGF-C is strikingly high in the developing nasal conchae.

5 The conchae are surrounded with a rich vascular plexus, important in nasal physiology as a source for the mucus produced by the epithelial cells and for warming inhaled air. It is suggested that VEGF-C is important in the formation of the conchal venous plexus at the mucous membranes, and that it may also regulate the permeability of the vessels needed for the secretion of nasal mucus. Possibly, VEGF-C and its derivatives,
10 and antagonists, could be used in the regulation of the turgor of the conchal tissue and mucous membranes and therefore the diameter of the upper respiratory tract, as well as the quantity and quality of mucus produced. These factors are of great clinical significance in inflammatory (including allergic) and infectious diseases of the upper respiratory tract. Accordingly, the invention contemplates the use of the materials of the invention, including
15 VEGF-C, Flt4, and their derivatives, in methods of diagnosing and treating inflammatory and infectious conditions affecting the upper respiratory tract, including nasal structures.

Example 31

Characterization of the exon-intron organization of the human VEGF-C gene

20 Two genomic DNA clones covering exons 1, 2, and 3 of the human VEGF-C gene were isolated from a human genomic DNA library using VEGF-C cDNA fragments as probes. In particular, a human genomic library in bacteriophage EMBL-3 lambda (Clontech) was screened using a PCR-generated fragment corresponding to nucleotides 629-746 of the human VEGF-C cDNA (SEQ ID NO: 7). One positive clone,
25 designated "lambda 3," was identified, and the insert was subcloned as a 14 kb *Xho*I fragment into the pBluescript II (pBSK II) vector (Stratagene). The genomic library also was screened with a labeled 130 bp *Not*I-*Sac*I fragment from the 5'-noncoding region of the VEGF-C cDNA (the *Not*I site is in the polylinker of the cloning vector; the *Sac*I site corresponds to nucleotides 92-97 of SEQ ID NO: 7). Two positive clones, designated
30 "lambda 5" and "lambda 8," were obtained. Restriction mapping analysis showed that

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clone lambda 3 contains exons 2 and 3, while clone lambda 5 contains exon 1 and the putative promoter region.

Three genomic fragments containing exons 4, 5, 6 and 7 were subcloned from a genomic VEGF-C P1 plasmid clone. In particular, purified DNA from a genomic P1 plasmid clone 7660 (Paavonen *et al.*, *Circulation*, 93: 1079-1082 (1996)) was used. *EcoRI* fragments of the P1 insert DNA were ligated into pBSK II vector. Subclones of clone 7660 which contained human VEGF-C cDNA homologous sequences were identified by colony hybridization, using the full-length VEGF-C cDNA as a probe. Three different genomic fragments were identified and isolated, which contained the remaining exons 4-7.

To determine the genomic organization, the clones were mapped using restriction endonuclease cleavage. Also, the coding regions and exon-intron junctions were partially sequenced. The result of this analysis is depicted in Figures 11A and 12. The sequences of all intron-exon boundaries (Fig. 11A, SEQ ID NOs: 24-35) conformed to the consensus splicing signals (Mount, *Nucl. Acids Res.*, 10: 459-472 (1982)). The length of the intron between exon 5 and 6 was determined directly by nucleotide sequencing and found to be 301 bp. The length of the intron between exons 2 and 3 was determined by restriction mapping and Southern hybridization and was found to be about 1.6 kb. Each of the other introns is over 10 kb in length.

A similar analysis was performed for the murine genomic VEGF-C gene. The sequences of murine VEGF-C intron-exon boundaries are depicted in Figure 11B and SEQ ID NOs: 36-47.

The restriction mapping and sequencing data indicated that the VEGF-C signal sequence and the first residues of the N-terminal propeptide are encoded by exon 1. The second exon encodes the carboxy-terminal portion of the N-terminal propeptide and the amino terminus of the VEGF homology domain. The most conserved sequences of the VEGF homology domain are distributed in exons 3 (containing 6 conserved cysteine residues) and 4 (containing 2 cys residues). The remaining exons encode cysteine-rich motifs of the type C-6X-C-10X-CRC (exons 5 and 7) and a fivefold repeated motif of type C-6X-B-3X-C-C-C, which is typical of a silk protein.

To further characterize the human VEGF-C gene promoter, the lambda 5 clone was further analyzed. Restriction mapping of this clone using a combination of

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single- and double-digestions and Southern hybridizations indicated that it includes: (1) an approximately 6 kb region upstream of the putative initiator ATG codon, (2) exon 1, and (3) at least 5 kb of intron I of the VEGF-C gene.

A 3.7 kb *Xba* I fragment of clone lambda 5, containing exon 1 and 5' and 3' flanking sequences, was subcloned and further analyzed. As reported previously, a major VEGF-C mRNA band migrates at a position of about 2.4 kb. Calculating from the VEGF-C coding sequence of 1257 bp and a 391 bp 3' noncoding sequence plus a polyA sequence of about 50-200 bp, the mRNA start site was estimated to be about 550-700 bp upstream of the translation initiation codon.

10 RNase protection assays were employed to obtain a more precise localization of the mRNA start site. The results of these experiments indicated that the RNA start site in the human VEGF-C gene is located 539 bp upstream of the ATG translational initiation codon.

To further characterize the promoter of the human VEGF-C gene, a
15 genomic clone encompassing about 2.4 kb upstream of the translation initiation site was isolated, and the 5' noncoding cDNA sequence and putative promoter region were sequenced. The sequence obtained is set forth in SEQ ID NO: 48. (The beginning of the VEGF-C cDNA sequence set forth in SEQ ID NO: 7 corresponds to position 2632 of
20 SEQ ID NO: 48.) Similar to what has been observed with the VEGF gene, the VEGF-C promoter is rich in G and C residues and lacks consensus TATA and CCAAT sequences. Instead, it has numerous putative binding sites (5'-GGGCGG-3' or 5'-CCGCCC-3') for Sp1, a ubiquitous nuclear protein that can initiate transcription of TATA-less genes. See Pugh and Tjian, *Genes and Dev.*, 5:105-119 (1991). In addition, sequences upstream of
25 the VEGF-C translation start site were found to contain frequent consensus binding sites for the AP-2 factor (5'-GCCN₃GCC-3') and binding sites for the AP-1 factor (5'-TKASTCA-3'). Binding sites for regulators of tissue-specific gene expression, like NFkB and GATA, are located in the distant part of VEGF-C promoter. This suggests that the cAMP-dependent protein kinase and protein kinase C, as activators of AP-2 transcription
30 factor [Curran and Fianza, *Cell*, 55:395-397 (1988)], mediate VEGF-C transcriptional regulation.

The VEGF-C gene is abundantly expressed in adult human tissues, such as heart, placenta, ovary and small intestine, and is induced by a variety of factors. Indeed, several potential binding sites for regulators of tissue-specific gene expression, like NFkB (5'-GGGRNTYYC-3') and GATA, are located in the distal part of the VEGF-C promoter.

5 For example, NFkB is known to regulate the expression of tissue factor in endothelial cells. Also, transcription factors of the GATA family are thought to regulate cell-type specific gene expression.

Unlike VEGF, the VEGF-C gene does not contain a binding site for the hypoxia-inducible factor, HIF-1 (Levy *et al.*, *J. Biol. Chem.*, 270: 13333-13340 (1995)).

10 This finding suggests that if the VEGF-C mRNA is regulated by hypoxia, the mechanism would be based mainly on the regulation of mRNA stability. In this regard, numerous studies have shown that the major control point for the hypoxic induction of the VEGF gene is the regulation of the steady-state level of mRNA. See Levy *et al.*, *J. Biol. Chem.*, 271: 2746-2753 (1996). The relative rate of VEGF mRNA stability and decay is
15 considered to be determined by the presence of specific sequence motifs in its 3' untranslated region (UTR), which have been demonstrated to regulate mRNA stability. (Chen and Shyu, *Mol. Cell Biol.*, 14: 8471-8482 (1994)). The 3'-UTR of the VEGF-C gene also contains a putative motif of this type (TTATTT), at positions 1873-1878 of SEQ ID NO: 7.

20 To identify DNA elements important for basal expression of VEGF-C in transfected cells, a set of luciferase reporter plasmids containing serial 5' deletions through the promoter region was constructed. Restriction fragments of genomic DNA containing 5' portions of the first exon were cloned into the polylinker of the pGL3 reporter vector (Promega) and confirmed by sequencing. About 10 μ g of the individual constructs in
25 combination with 2 μ g of pSV2- β -galactosidase plasmid (used as a control of transfection efficiency) were transfected into HeLa cells using the calcium phosphate-mediated transfection method. Two days after transfection, the cells were harvested and subjected to the luciferase assay. The luciferase activity was normalized to that of the pGL3 control vector driven by SV40 promoter/enhancer.

30 As depicted in Fig. 3, the 5.5 kb *XhoI*-*RsrII* fragment of clone lambda 5 gave nearly 9-fold elevated activity when compared with a promoterless vector. Deletion of a 5' *XhoI*-*HindIII* fragment of 2 kb had no effect on the promoter activity. The activity

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of the 1.16 kb *XbaI-RsrII* fragment was about twice that of the pGL3 basic vector, while the activity of the same fragment in the reverse orientation was at background level. Further deletion of the *XbaI-SacI* fragment caused an increase in the promoter activity, suggesting the presence of silencer elements in the region from -1057 to -199 (i.e., 199 to 5 1057 bp upstream from the transcription initiation site). The shortest fragment (*SacII-RsrII*) yielded only background activity, which was consistent with the fact that the mRNA initiation site was not present in this construct.

To determine whether further sequences in the first exon of human VEGF-C are important for basal expression, an *RsrII* fragment spanning nucleotides 214-495 (i.e., 10 214-495 bp downstream from the transcription initiation site) was subcloned in between of *XbaI-RsrII* fragment and the luciferase reporter gene. Indeed, the obtained construct showed an 50 % increase in activity when compared with the *XbaI-RsrII* construct.

The VEGF gene has been shown to be up-regulated by a number of stimuli including serum derived growth factors. To find out whether the VEGF-C gene also can 15 be stimulated by serum, RNA from serum-starved and serum-stimulated HT1080 cells was subjected to primer extension analysis, which demonstrated that VEGF-C mRNA is up-regulated by serum stimulation.

Additional serum stimulation experiments indicated that the serum stimulation leads to increased VEGF-C promoter activity. Cells were transfected as 20 described above and 24 h after transfection changed into medium containing 0.5% bovine serum albumin. Cells were then stimulated with 10 % fetal calf serum for 4 hours and analyzed. The *XbaI-RsrII* promoter construct derived from lambda 5 yielded a twofold increased activity upon serum stimulation, while the same fragment in the reverse orientation showed no response. All other promoter constructs also showed up- 25 regulation, ranging from 1.4 to 1.6 fold (Fig. 3).

Example 32

Identification of a VEGF-C splice variant

As reported in Example 16, a major 2.4 kb VEGF-C mRNA and smaller amounts of a 2.0 kb mRNA are observable. To clarify the origin of these RNAs, several 30 additional VEGF-C cDNAs were isolated and characterized. A human fibrosarcoma cDNA library from HT1080 cells in the lambda gt11 vector (Clontech, product

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#HL1048b) was screened using a 153 bp human VEGF-C cDNA fragment as a probe as described in Example 10. See also Joukov *et al.*, *EMBO J.*, 15:290-298 (1996). Nine positive clones were picked and analyzed by PCR amplification using oligonucleotides 5'-CACGGCTTATGCAAGCAAAG-3' (SEQ ID NO: 49) and
5 5'-AACACAGTTTTCCATAATAG-3' (SEQ ID NO: 50) These oligonucleotides were selected to amplify the portion of the VEGF-C cDNA corresponding to nucleotides 495-1661 of SEQ ID NO: 7. PCR was performed using an annealing temperature of 55°C and 25 cycles.

The resultant PCR products were electrophoresed on agarose gels. Five
10 clones out of the nine analyzed generated PCR fragments of the expected length of 1147 base pairs, whereas one was slightly shorter. The shorter fragment and one of the fragments of expected length were cloned into the pCRTMII vector (Invitrogen) and analyzed by sequencing. The sequence revealed that the shorter PCR fragment had a deletion of 153 base pairs, corresponding to nucleotides 904 to 1055 of SEQ ID NO: 7.
15 These deleted bases correspond to exon 4 of the human and mouse VEGF-C genes, schematically depicted in Figs. 13A and 13B. Deletion of exon 4 results in a frameshift, which in turn results in a C-terminal truncation of the full-length VEGF-C precursor, with fifteen amino acid residues translated from exon 5 in a different frame than the frame used to express the full-length protein. Thus, the C-terminal amino acid sequence of the
20 resulting truncated polypeptide would be --Leu (181)-Ser-Lys-Thr-Val-Ser-Gly-Ser-Glu-Gln-Asp-Leu-Pro-His-Glu-Leu-His-Val-Glu(199) (SEQ ID NO: 51). The polypeptide encoded by this splice variant would not contain the C-terminal cleavage site of the VEGF-C precursor. Thus, a putative alternatively spliced RNA form lacking conserved exon 4 was identified in HT-1080 fibrosarcoma cells and this form is predicted to encode a
25 protein of 199 amino acid residues, which could be an antagonist of VEGF-C.

Example 33

VEGF-C is similarly processed in different cell cultures in vitro

To study whether VEGF-C is similarly processed in different cell types, 293 EBNA cells, COS-1 cells and HT-1080 cells were transfected with wild type human
30 VEGF-C cDNA and labelled with Pro-Mix™ as described in Example 22. The conditioned media from the cultures were collected and subjected to immunoprecipitation using

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antiserum 882 (described in Example 21, recognizing a peptide corresponding to amino acids 104-120 of SEQ ID NO: 8). The immunoprecipitated polypeptides were separated via SDS-PAGE, and detected via autoradiography. The major form of secreted recombinant VEGF-C observed from all cell lines tested is a 29/32 kD doublet. These two polypeptides are bound to each other by disulfide bonds, as described in Example 22. A less prominent band of approximately 21 kD also was detected in the culture media. Additionally, a non-processed VEGF-C precursor of 63 kDa was observed. This form was more prominent in the COS-1 cells, suggesting that proteolytic processing of VEGF-C in COS cells is less efficient than in 293 EBNA cells. Endogenous VEGF-C (in non-transfected cells) was not detectable under these experimental conditions in the HT-1080 cells, but was readily detected in the conditioned medium of the PC-3 cells. Analysis of the subunit polypeptide sizes and ratios in PC-3 cells and 293 EBNA cells revealed strikingly similar results: the most prominent form was a doublet of 29/32 kDa and a less prominent form the 21 kD polypeptide. The 21 kD form produced by 293 EBNA cells was not recognized by the 882 antibody in the Western blot, although it is recognized when the same antibody is used for immunoprecipitation (see data in previous examples). As reported in Example 21, cleavage of the 32 kD form in 293 EBNA cells occurs between amino acid residues 111 and 112 (SEQ ID NO: 8), downstream of the cleavage site in PC-3 cells (between residues 102 and 103). Therefore, the 21 kD form produced in 293 EBNA cells does not contain the complete N-terminal peptide used to generate antiserum 882. In a related experiment, PC-3 cells were cultured in serum-free medium for varying periods of time (1 - 8 days) prior to isolation of the conditioned medium. The conditioned medium was concentrated using a Centricon device (Amicon, Beverly, USA) and subjected to Western blotting analysis using antiserum 882. After one day of culturing, a prominent 32 kD band was detected. Increasing amounts of a 21-23 kD form were detected in the conditioned media from 4 day and 8 day cultures. The diffuse nature of this polypeptide band, which is simply called the 23 kD polypeptide in example 5 and several subsequent examples, is most likely due to a heterogeneous and variable amount of glycosylation. These results indicate that, initially, the cells secrete a 32 kD polypeptide, which is further processed or cleaved in the medium to yield the 21-23 kD form. The microheterogeneity of this polypeptide band would then arise from the variable glycosylation degree and, from microheterogeneity of the processing cleavage sites, such

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as obtained for the amino terminus in PC-3 and 293 EBNA cell cultures. The carboxyl terminal cleavage site could also vary, examples of possible cleavage sites would be between residues 225-226, 226-227 and 227-228 as well as between residues 216-217. Taken together, these data suggest the possibility that secreted cellular protease(s) are responsible for the generation of the 21-23 kD form of VEGF-C from the 32 kD polypeptide. Such proteases could be used in vitro to cleave VEGF-C precursor proteins in solution during the production of VEGF-C, or used in cell culture and in vivo to release biologically active VEGF-C.

Example 34

10 Differential binding of VEGF-C forms by the extracellular domains of VEGFR-3 and VEGFR-2

In two parallel experiments, 293 EBNA cells were transfected with a construct encoding recombinant wild type VEGF-C or a construct encoding VEGF-C Δ N Δ CHis (Example 28) and about 48 hours after transfection, metabolically labelled with 15 Pro-Mix™ as described in previous examples. The media were collected from mock-transfected and transfected cells and used for receptor binding analyses.

Receptor binding was carried out in binding buffer (PBS, 0.5% BSA, 0.02% Tween 20, 1 microgram/ml heparin) containing approximately 0.2 microgram of either (a) a fusion protein comprising a VEGFR-3 extracellular domain fused to an 20 immunoglobulin sequence (VEGFR-3-Ig) or (b) a fusion protein comprising VEGFR-2 extracellular domain fused to an alkaline phosphatase sequence (VEGFR-2-AP; Cao *et al.*, *J. Biol. Chem.* 271:3154-62 (1996)). As a control, similar aliquots of the 293 EBNA conditioned media were mixed with 2 μ l of anti-VEGF-C antiserum (VEGF-C IP).

After incubation for 2 hours at room temperature, anti-VEGF-C antibodies 25 and VEGFR-3-Ig protein were adsorbed to protein A-sepharose (PAS) and VEGFR-2-AP was immunoprecipitated using anti-AP monoclonal antibodies (Medix Biotech, Genzyme Diagnostics, San Carlos, CA, USA) and protein G-sepharose. Complexes containing VEGF-C bound to VEGFR-3-Ig or VEGFR-2-AP were washed three times in binding buffer, twice in 20 mM Tris-HCl (pH 7.4) and VEGF-C immunoprecipitates were washed 30 three times in RIPA buffer and twice in 20 mM tris-HCl (pH 7.4) and analyzed via SDS-PAGE under reducing and nonreducing conditions. As a control, the same media were

precipitated with antiAP and protein G-sepharose (PGS) or with PAS to control for possible nonspecific adsorption.

These experiments revealed that VEGFR-3 bound to both the 32/29 kD and 21-23 kD forms of recombinant VEGF-C, whereas VEGFR-2 bound preferentially to the 21-23 kD component from the conditioned media. In addition, small amounts of 63 kD and 52 kD VEGF-C forms were observed binding with VEGFR-3. Further analysis under nonreducing conditions indicates that a great proportion of the 21-23 kD VEGF-C bound to either receptor does not contain interchain disulfide bonds. These findings reinforce the results that VEGF-C binds VEGFR-2. This data suggests a utility for recombinant forms of VEGF-C which are active towards VEGFR-3 only or which are active towards both VEGFR-3 and VEGFR-2. On the other hand, these results, together with the results in Example 28, do not eliminate the possibility that the 32/29 kD dimer binds VEGFR-3 but does not activate it. The failure of the 32/29 kD dimer to activate VEGFR-3 could explain the finding that conditioned medium from the N-His VEGF-C transfected cells induced a less prominent tyrosine phosphorylation of VEGFR-3 than medium from VEGF-C Δ N Δ CHis transfected cells, even though expression of the former polypeptide was much higher. Stable VEGF-C polypeptide mutants that bind to a VEGF-C receptor but fail to activate the receptor are useful as VEGF-C antagonists.

Example 35

Discovery of VEGF-C analogs that selectively bind to and activate VEGFR-3, but not VEGFR-2

To further identify the cysteine residues of VEGF-C that are critical for retaining VEGF-C biological activities, an additional VEGF-C mutant, designated VEGF-C Δ N Δ CHisC156S, was synthesized, in which the cysteine residue at position 156 of the 419 amino acid VEGF-C precursor (SEQ ID NO: 8; Genbank accession number X94216) was replaced with a serine residue.

The mutagenesis procedure was carried out using the construct of VEGF-C Δ N Δ CHis (see Example 28), cloned in the pALTER vector, and the *Altered sites II in vitro mutagenesis system* of Promega. An oligonucleotide 5'-GACGGACACAGATGGAGGTTTAAAG-3' (SEQ ID NO: 52) was used to introduce the desired mutation in the cDNA encoding VEGF-C Δ N Δ CHis. The resulting mutated

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VEGF-C cDNA fragment was subcloned into the *HindIII*/*NotI* sites of the pREP-7 vector (Invitrogen), and the final construct was re-sequenced to confirm the C156S mutation.

The resultant clone has an open reading frame encoding amino acids 103-225 of SEQ ID NO: 8 (with a serine codon at position 156), and further encoding a 6xHis tag.

5 The wildtype VEGF-C cDNA and three VEGF-C mutant constructs (VEGF-C R226,227S, VEGF-C Δ N Δ CHis, and VEGF-C Δ N Δ CHisC156S) were used to transfect 293 EBNA cells, which were subcultured 16 hours after transfection. About 48 hours after transfection, the media were changed to DMEM/0.1% BSA, and incubation in this medium was continued for an additional 48 hours. The resultant conditioned media
10 were concentrated 30-fold using *Centriprep-10* (Amicon), and the amount of VEGF-C in the media was analyzed by Western blotting using the anti-VEGF-C antiserum 882 for immunodetection. Different amounts of the recombinant VEGF-C Δ N Δ CHis, purified from a yeast expression system, were analyzed in parallel as reference samples to measure and equalize the VEGF-C concentrations in the conditioned media. The conditioned
15 medium from mock-transfected cells was used to dilute the VEGF-C conditioned media to achieve equal concentrations.

 An aliquot of the transfected cells were metabolically labelled for 6 hours with 100 microcuries/ml of the PRO-MIX™ L- $[^{35}\text{S}]$ *in vitro* cell labelling mix (Amersham). The conditioned media were collected, and binding of the radioactively
20 labelled VEGF-C proteins to the extracellular domains of VEGFR-3 and VEGFR-2 was analyzed using recombinantly produced VEGFR-3EC-Ig and VEGFR-2EC-Ig constructs (containing seven and three Ig loops of the extracellular domains of the respective receptors, fused to an immunoglobulin heavy chain constant region).

 All processed VEGF-C forms secreted to the culture medium bound to
25 VEGFR-3EC domain, with preferential binding of the 21 kDa form. When present at high concentrations, the VEGF-C forms of 58 kDa and 29/31 kDa bound to some extent non-specifically to protein A Sepharose.

 The VEGFR-2EC domain preferentially bound the mature 21 kDa form of wildtype VEGF-C and VEGF-C Δ N Δ CHis. Significantly, VEGF-C Δ N Δ CHisC156S failed
30 to bind the VEGFR2-EC.

 Next, the ability of the above-described VEGF-C polypeptides to compete with the ^{125}I -VEGF-C Δ N Δ CHis for binding to VEGFR-2 and VEGFR-3 was analyzed.

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Scatchard analysis using VEGF-C Δ CANHis provided indications of the VEGF-C binding affinity for VEGFR-3 ($K_D=135$ pM) and VEGFR-2 ($K_D=410$ pM). Ten micrograms of the purified yeast VEGF-C Δ NACHis was labeled using 3 mCi of Iodine-125, carrier-free (Amersham), and an Iodo-Gen Iodination Reagent (Pierce), according to the standard protocol of Pierce. The resulting specific activity of the labeled VEGF-C Δ NACHis was 1.25×10^5 cpm/ng.

To study receptor binding, PAE/VEGFR-2 and PAE/VEGFR-3 cells were seeded into 24-well tissue culture plates (Nunc), which had been coated with 2% gelatin in PBS. The 125 I-VEGF-C Δ NACHis (2×10^5 cpm) and different amounts of media containing equal concentrations of the non-labeled VEGF-C (wildtype and mutants) were added to each plate in Ham's F12 medium, containing 25 mM HEPES (pH 8.0), 0.1% BSA, and 0.1% NaN_3 . The binding was allowed to proceed at room temperature for 90 minutes. The plates were then transferred onto ice and washed three times with ice-cold PBS containing 0.1% BSA. The cells were then lysed in 1 M NaOH, the lysates were collected, and the radioactivity was measured using a γ -counter. Binding in the presence of VEGF-C-containing conditioned medium was calculated as a percentage of binding observed in parallel control studies wherein equal volumes of medium from mock-transfected cells were used instead of VEGF-C conditioned media.

As shown in Fig. 4, left panel, all VEGF-C mutants displaced 125 I-VEGF-CANACHis from VEGFR-3. The efficiency of displacement was as follows: VEGF-CANACHisC156S > VEGF-CANACHis > wildtype VEGF-C > VEGF-CR226,227S. These results indicate that enhanced binding to VEGFR-3 was obtained upon "recombinant maturation" of VEGF-C. Recombinant VEGF165 failed to displace VEGF-C from VEGFR-3.

VEGF, VEGF-CANACHis, and wildtype VEGF-C all efficiently displaced labeled VEGF-CANACHis from VEGFR-2, with VEGF-CANACHis being more potent when compared to wildtype VEGF-C (Fig. 4, right panel). The non-processed VEGF-C R226,227S showed only weak competition of 125 I-VEGF-CANACHis.

Surprisingly, VEGF-CANACHisR156S failed to displace VEGF-CANACHis from VEGFR-2, thus confirming the above described results obtained using a soluble extracellular domain of VEGFR-2.

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The ability of the above mentioned VEGF-C forms to stimulate tyrosine phosphorylation of VEGFR-3 and VEGFR-2 was also investigated. Importantly, identical dilutions of the conditioned media were used for these experiments and for the competitive binding experiments described above. A Western blot analysis of the conditioned media using anti-VEGF-C antiserum 882 was performed to confirm the approximately equal relative amounts of the factors present.

The stimulation of VEGFR-3 and VEGFR-2 autophosphorylation by the different VEGF-C forms in general correlated with their binding properties, as well as with the degree of "recombinant processing" of VEGF-C. The VEGF-CAN Δ CHisC156S appeared to be at least as potent as VEGF-CAN Δ CHis in stimulating VEGFR-3 autophosphorylation. VEGF-CAN Δ CHis showed a higher potency when compared to wildtype VEGF-C in its ability to stimulate tyrosine autophosphorylation of both VEGFR-2 and VEGFR-3. The VEGF-CR226,227S conditioned medium possessed a considerably weaker effect on autophosphorylation of VEGFR-3, and almost no effect on VEGFR-2 autophosphorylation.

Stimulation of VEGFR-2 tyrosine phosphorylation by VEGF-CAN Δ CHisC156S did not differ from that of conditioned medium from the mock transfected cells, thus confirming the lack of VEGFR-2-binding and VEGFR-2-activating properties of this mutant.

The ability of VEGF-C Δ N Δ CHisC156S to alter vascular permeability *in vivo* was analyzed using the Miles assay (see Example 29). The recombinant VEGF-C forms assayed (Δ N Δ CHis, Δ N Δ CHisC156S) were produced by 293 cells, purified from conditioned media using Ni-NTA Superflow resin (QIAGEN) as previously described, and pretreated with 15 μ g/ml of anti-human VEGF neutralizing antibody (R&D systems) to neutralize residual amounts of co-purified, endogenously produced VEGF. Eight picomoles of the various VEGF-C forms, as well as 2 pmol of recombinant human VEGF165 (R&D systems) and approximately 2 pmol of VEGF165 from the conditioned medium which were either non-treated or pretreated with the above mentioned VEGF-neutralizing antibody were injected subcutaneously to the back region of a guinea pig. The area of injection was analyzed 20 minutes after injections. Both VEGF and VEGF-C Δ N Δ CHis caused increases in vascular permeability, whereas Δ N Δ CHisC156S did not affect vascular permeability. The neutralizing antibody completely blocked permeability

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activity of VEGF but did not affect VEGF-C activity. Residual permeability activity observed for the VEGF-containing conditioned medium even after its treatment with VEGF neutralizing antibody was presumably caused by permeability factors other than VEGF that are produced by 293 cells.

5 In yet another assay, the ability of VEGF-C Δ N Δ CHis and VEGF-C Δ N Δ CHisC156S to stimulate migration of bovine capillary endothelial cells in a collagen gel was analyzed. The Δ N Δ CHis form dose-dependently stimulated migration, whereas the Δ N Δ CHisC156S form had no significant activity in the assay.

The Miles assay also was used to assay the ability of VEGF-C R226,227S
10 (8 pM, pretreated with anti-VEGF antibody) to induce vascular permeability. The results indicated that the ability of VEGF-C R226,227S to induce vascular permeability was much weaker when compared to wildtype and Δ N Δ CHis forms of VEGF-C. Collectively, this Miles assay data is consistent with the VEGFR-2 binding and autophosphorylation data described above, and indicates that VEGF-C effect on vascular permeability is mediated
15 via VEGFR-2.

Mitogenic signals from growth factor receptors are frequently relayed via the extracellular signal regulated kinases/mitogen activated protein kinases (ERK/MAPK) pathway into the nucleus. Purified recombinant VEGF-C Δ N Δ CHis and VEGF-C Δ N Δ C156S produced by a Pichia expression system were used to determine MAPK
20 pathway activation of cells expressing either VEGFR-2 or VEGFR-3. The growth factor treated cells were lysed, and activated MAPK was detected using Western blotting with antibodies against the phosphorylated forms of ERK1 and ERK2. At a concentration of 100 ng/ml, VEGF-C Δ N Δ CHis showed rapid activation of the ERK1 and ERK2 MAPK in both VEGFR-2- and VEGFR-3-expressing cells. In contrast, VEGF-C Δ N Δ C156S
25 activated ERK1 and ERK2 exclusively in the VEGFR-3-expressing cells. At the concentrations used, both VEGF-C Δ N Δ CHis and VEGF-C Δ N Δ C156S appeared to be equally potent in activating the MAPK through VEGFR-3. The amounts of total MAPK protein were confirmed to be similar in the treated and untreated cells, as shown by staining of the filter with p44/p42 MAPK antibodies made against a synthetic peptide of
30 rat p42.

The foregoing data indicates that proteolytic processing of VEGF-C results in an increase in its ability to bind and to activate VEGFR-3 and VEGFR-2. Non-

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processed VEGF-C is a ligand and an activator of preferentially VEGFR-3, while the mature 21/23 kDa VEGF-C form is a high affinity ligand and an activator of both VEGFR-3 and VEGFR-2.

Moreover, replacement of the cysteine residue at position 156 (of prepro-VEGF-C, SEQ ID NO: 8) creates a selective ligand and activator of VEGFR-3. This alteration inactivates the ability of processed VEGF-C to bind to VEGFR-2 and to activate VEGFR-2. Importantly, it is believed that the elimination of the cysteine at position 156 is the alteration responsible for this unexpected alteration in VEGF-C selectivity, and not the substitution of a serine per se. It is expected that replacement of the cysteine at position 156 with other amino acids, or the mere deletion of this cysteine, will also result in VEGF-C analogs having selective biological activity with respect to VEGFR-3. All such replacement and deletion analogs (collectively referred to as VEGF-C ΔC_{156} polypeptides) are contemplated as aspects of the present invention. Thus, "VEGF-C ΔC_{156} polypeptides" of the invention derived from human VEGF-C include polypeptides depicted in SEQ ID NO: 58, fragments of those polypeptides (especially fragments having an amino terminus anywhere between residues 102 and 161 of SEQ ID NO: 58 and a carboxy-terminus anywhere between residues 210 and 228 of SEQ ID NO: 58). "VEGF-C ΔC_{156} polypeptides" of the invention also include the corresponding polypeptides derived from murine, quail, and other wildtype VEGF-C polypeptides.

VEGF-C polypeptides that have the C156S mutation (or functionally equivalent mutations at position 156) and that retain biological activity with respect to VEGFR-3, such as VEGF-C $\Delta N\Delta C_{156}H_{156}S$, are useful in all of the same manners described above for wildtype VEGF-C proteins and biologically active fragments thereof where VEGFR-3 stimulation is desired. It is contemplated that most biologically active VEGF-C fragments and processing variants, including but not limited to the biologically active fragments and variants identified in preceding examples, will retain VEGF-C biological activity (as mediated through VEGFR-3) when a ΔC_{156} mutation is introduced. All such biologically active VEGF-C ΔC_{156} polypeptides are intended as an aspect of the present invention.

Moreover, VEGF-C forms containing the C156S mutation or equivalent mutations can be used to distinguish those effects of VEGF-C mediated via VEGFR-3 and VEGFR-2 from those obtained via only VEGFR-3. The ability of such VEGF-C

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polypeptides to selectively stimulate VEGFR-3 are also expected to be useful in clinical practice, it being understood that selectivity of a pharmaceutical is highly desirable in many clinical contexts. For example, the selectivity of VEGF-C ΔC_{156} polypeptides for VEGFR-3 binding suggests a utility for these peptides to modulate VEGF-C biological activities mediated through VEGFR-3, without significant concomitant modulation of blood vessel permeability or other VEGF-C activities that are modulated through VEGFR-2.

The data presented herein also indicates a utility for ΔC_{156} polypeptides that are capable of binding VEGFR-3, but that do not retain biological activity mediated through VEGFR-3. Specifically, such forms are believed to be capable of competing with wildtype VEGF-C for binding to VEGFR-3, and are therefore contemplated as molecules that inhibit VEGF-C-mediated stimulation of VEGFR-3. Because of the ΔC_{156} alteration, such polypeptides (especially covalent or noncovalent dimers of such polypeptides) are not expected to bind VEGFR-2. Thus, certain ΔC_{156} polypeptides and polypeptide dimers are expected to have utility as selective inhibitors of VEGF-C biological activity mediated through VEGFR-3 (i.e., without substantially altering VEGF-C mediated stimulation of VEGFR-2).

In another embodiment of the invention, heterodimers comprising a biologically active VEGF-C polypeptide in association with a ΔC_{156} polypeptide are contemplated. It is contemplated that such heterodimers can be formed *in vitro*, as described below in Example 37, or formed *in vivo* with endogenous VEGF-C following administration of a ΔC_{156} polypeptide. Such heterodimers are contemplated as modulators of VEGF-C mediated effects in cells where the biological effects of VEGF-C are mediated through VEGFR-2/VEGFR-3 heterodimers. VEGF-C ΔC_{156} polypeptides in homodimers or in heterodimers with wt VEGF-C might selectively inhibit the ability of the latter to induce VEGF-like effects, particularly to increase the vascular permeability.

Replacement of the second and/or the fourth of the eight conserved cysteine residues of VEGF abolishes VEGF dimer formation and VEGF biological activity. The analogous effect was investigated for VEGF-C, wherein the cysteines at positions 156 and 165 of SEQ ID NO: 8 correspond to the second and fourth conserved cysteines. No homodimers were obtained when VEGF-C $\Delta N\Delta C$ HisC156,165S (i.e., Cys₁₅₆ and Cys₁₆₅ both replaced with serine residues) or in VEGF-C $\Delta N\Delta C$ HisC165S were chemically crosslinked. On the other hand, about half of both crosslinked VEGF-C $\Delta N\Delta C$ His and

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VEGF- Δ N Δ CHisC156S migrated as dimers. This data indicates that VEGF- Δ N Δ CHisC156S forms homodimers. Moreover, unlike VEGF- Δ N Δ CHis, which forms preferentially non-covalently bound dimers, a fraction of VEGF- Δ N Δ CHisC156S was disulfide bonded, as detected by SDS-PAGE in non-reducing conditions. In receptor binding studies (using procedures such as those described above), the C165S and C156,165S forms were both unable to bind VEGFR-3 or VEGFR-2. Collectively, these data suggest that homodimerization is required for VEGFR-3 activation by VEGF-C, and indicate that the inability of Δ N Δ C156S to activate VEGFR-2 and to induce VEGF-like effects is not due to an inability of this mutant to form homodimers.

10

Example 36**Utility for VEGF-C in promoting myelopoiesis**

The effects of VEGF-C on hematopoiesis were also analyzed. Specifically, leukocytes populations were analyzed in blood samples taken from the F1 transgenic mice described in Example 29, and from their non-transgenic littermates. Leukocyte population data from these mice and from non-transgenic FVB-NIH control mice (i.e., the strain used to generate the transgenic mice) are set forth in the tables below.

20

FVB/NIH MICE					
Cell Type	male 5.5 months	male 5.5 months	female 9.5 months	male 9.5 months	mean $\pm\sigma$
Lymphocytes	72.20%	82.17%	84.25%	74.25%	78.22 \pm 5.10
Neutrophils	23.00%	15.17%	14.25%	22.25%	18.67\pm3.98
Monocytes	0.65%	1.00%	0.25%	0.50%	0.60 \pm 0.27
Eosinophils	2.15%	1.70%	1.25%	3.00%	2.03 \pm 0.65
Basophils	0.00%	0.00%	0.00%	0.00%	0 \pm 0

VEGF-C TRANSGENIC MICE				
Cell Type	male 2 months	male 3.5 months	male 7 months	mean $\pm\sigma$
Lymphocytes	41.3%	41.50%	18.70%	33.83 \pm 10.70
Neutrophils	55.3%	53.80%	80.17%	63.09\pm12.09
Monocytes	2.16%	1.30%	0.67%	1.38 \pm 0.61
Eosinophils	1.17%	3.50%	.50%	1.72 \pm 1.29
Basophils	0.00%	0.00%	0.00%	0 \pm 0

VEGF-C NEGATIVE CONTROL MICE (NON-TRANSGENIC LITTERMATES OF VEGF-C TRANSGENIC MICE)					
Cell Type	male 2 months	male 2 months	male 3.5 months	male 7 months	mean $\pm\sigma$
Lymphocytes	89.00%	67.50%	91.00%	71.30%	79.7 \pm 10.41
Neutrophils	7.75%	23.00%	7.00%	23.75%	15.38\pm8.01
Monocytes	1.50%	0.50%	0.83%	0.75%	0.90 \pm 0.37
Eosinophils	1.50%	9.00%	0.67%	4.00%	3.79 \pm 3.25
Basophils	0.00%	0.00%	0.50%	0.50%	0.25 \pm 0.25

As the foregoing data indicates, the overexpression of VEGF-C in the skin of the transgenic mice correlates with a distinct alteration in leukocyte populations. Notably, the measured populations of neutrophils were markedly increased in the transgenic mice. One explanation for the marked increase in neutrophils is a myelopoietic activity attributable to VEGF-C. A VEGF-C influence on leukocyte trafficking in and out of tissues also may effect observed neutrophil populations. Fluorescence-activated cell sorting analysis, performed on isolated human bone marrow and umbilical cord blood CD34-positive hematopoietic cells, demonstrated that a fraction of these cells are positive for Flt4 (VEGFR-3). Thus, the VEGF-C effect on myelopoiesis may be exerted through this VEGFR-3-positive cell population and its receptors. In any case, the foregoing data indicates a use for VEGF-C polypeptides to increase granulocyte (and, in particular,

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neutrophil) counts in human or non-human subjects, i.e., in order to assist the subject fight infectious diseases. The exploitation of the myelopoietic activity of VEGF-C polypeptides is contemplated both *in vitro* (i.e., in cell culture) and *in vivo*, as a sole myelopoietic agent and in combination with other effective agents (e.g., granulocyte colony stimulating factor 5 (G-CSF)).

Additional studies of the myelopoietic effect of VEGF-C, using VEGF-C mutants (e.g., VEGF-C ΔC_{156} polypeptides, VEGF-C $\Delta N\Delta CHis$, VEGF-C R226,227S) having altered VEGFR-2 binding affinities, will elucidate whether this effect is mediated through VEGFR-2, VEGFR-3, or both receptors, for example. The results of such analysis will be useful in determining which VEGF-C mutants have utility as myelopoietic agents and which have utility as agents for inhibiting myelopoiesis.

Example 37

Generation of Heterodimers consisting of members of the VEGF family of growth factors

Both naturally-occurring and recombinantly-produced heterodimers of polypeptides of the PDGF/VEGF family of growth factors have been shown to exist in nature and possess mitogenic activities. See, e.g., Cao *et al.*, *J. Biol. Chem.*, 271:3154-62 (1996); and DiSalvo, *et al.*, *J. Biol. Chem.*, 270:7717-7723 (1995). Heterodimers comprising a VEGF-C polypeptide may be generated essentially as described In Cao *et al.* (1996), using recombinantly produced VEGF-C polypeptides, such as the VEGF-C polypeptides described in the preceding examples. Briefly, a recombinantly produced VEGF-C polypeptide is mixed at an equimolar ratio with another recombinantly produced polypeptide of interest, such as a VEGF, VEGF-B, PlGF, PDGF α , PDGF β , or *c-fos* induced growth factor polypeptide. (See, e.g., Cao *et al.* (1990); Collins *et al.*, *Nature*, 316:748-750 (1985) (PDGF- β , GenBank Acc. No. X02811); Claesson-Welsh *et al.*, *Proc. Natl. Acad. Sci. USA*, 86(13):4917-4921 (1989) (PDGF- α , GenBank Acc. No. M22734); Claesson-Welsh *et al.*, *Mol. Cell. Biol.* 8:3476-3486 (1988) (PDGF- β , GenBank Acc. No. M21616); Olofsson *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 93:2576-2581 (1996) (VEGF-B, GenBank Acc. No. U48801); Maglione *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 88(20):9267-9271 (1996) (PlGF, GenBank Acc. No. X54936); Heldin *et al.*, *Growth Factors*, 8:245-252 (1993); Folkman, *Nature Med.*, 1:27-31 (1995); Friesel *et al.*, *FASEB J.*, 9:919-25

(1995); Mustonen *et al.*, *J. Cell. Biol.*, 129:895-98 (1995); Orlandini, S., *Proc. Natl. Acad. Sci. USA*, 93(21):11675-11680 (1996); and others cited elsewhere herein. The mixed polypeptides are incubated in the presence of guanidine-HCl and DTT. The thiol groups are then protected with S-sulfonation, and the protein is dialyzed overnight, initially
5 against urea/glutathione-SH, glutathione-S-S-glutathione, and subsequently against 20 mM Tris-HCl.

In a preferred embodiment, a variety of differently processed VEGF-C forms and VEGF-C variants and analogs, such as the ones described in the preceding examples, are employed as the VEGF-C polypeptide used to generate such heterodimers.
10 Thereafter, the heterodimers are screened to determine their binding affinity with respect to receptors of the VEGF/PDGF family (especially VEGFR-1, VEGFR-2, and VEGFR-3), and their ability to stimulate the receptors (e.g., assaying for dimer-stimulated receptor phosphorylation in cells expressing the receptor of interest on their surface). The binding assays may be competitive binding assays such as those described herein and in the art. In
15 the initial binding assays, recombinantly produced proteins comprising the extracellular domains of receptors are employable, as described in preceding examples for VEGFR-2 and VEGFR-3. Heterodimers that bind and stimulate receptors are useful as recombinant growth factor polypeptides. Heterodimers that bind but do not stimulate receptors are useful as growth factor antagonists. Heterodimers that display agonistic or antagonistic
20 activities in the screening assays are further screened using, e.g., endothelial cell migration assays, vascular permeability assays, and *in vivo* assays. It will also be apparent from the preceding examples that dimers comprising two VEGF-C polypeptides (i.e., dimers of identical VEGF-C polypeptides as well as dimers of different VEGF-C polypeptides) are advantageously screened for agonistic and antagonistic activities using the same assays.

25 In one preferred embodiment, VEGF-C ΔC_{156} polypeptide is employed to make the dimers. It is anticipated that agonists and antagonists comprising a VEGF-C ΔC_{156} polypeptide will have increased specificity for stimulating and inhibiting VEGFR-3, without concomitant stimulation or inhibition of VEGFR-2.

In another preferred embodiment, VEGF-C polypeptides wherein the C-
30 terminal proteolytic cleavage site has been altered to reduce or eliminate C-terminal processing (e.g. VEGF-C R226,227S) is employed to make dimers for screening for inhibitory activity.

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In yet another preferred embodiment, VEGF-C polypeptides comprising amino-terminal fragments (e.g., the VEGF-C 15 kD form described herein) of VEGF-C are employed to make dimers.

It is further contemplated that inactivation of only one polypeptide chain in a dimer could be enough to generate an inhibitory molecule, which is demonstrated e.g., by the generation of PDGF inhibitory mutant as reported in Vassbotn, *Mol. Cell. Biol.*, 13:4066-4076 (1993). Therefore, in one embodiment, inhibition is achieved by expression *in vivo* of a polynucleotide (e.g., a cDNA construct) encoding the heterodimerization partner which is unable to bind (or binds inefficiently) to the receptor, or by direct administration of that monomer in a pharmaceutical composition.

Example 38

Formation and Screening of Useful Recombinant VEGF/VEGF-C genes and polypeptides

Amino acid sequence comparison reveals that mature VEGF-C bears structural similarity to VEGF121 [Tischer *et al.*, *J. Biol. Chem.*, 266(18):11947-54 (1991)], with certain noteworthy structural differences. For example, mature VEGF-C contains an unpaired cysteine (position 137 of SEQ ID NO: 8) and is able to form non-covalently bonded polypeptide dimers. In one embodiment of the invention, a VEGF analog is created wherein the unpaired cysteine residue from mature VEGF-C is introduced at an analogous position of VEGF (e.g., introduced at Leu₅₈ of the human VEGF165 precursor (Fig. 2, Genbank Acc. No. M32977) to generate a VEGF^{+cys} mutant designated VEGF L58C). Such an alteration is introduced into the VEGF165 coding sequence using site-directed mutagenesis procedures known in the art, such as the procedures described above in preceding examples to generate various VEGF-C mutant forms. This VEGF^{+cys} mutant is recombinantly expressed and is screened (alone and as a heterodimer with other VEGF and VEGF-C forms) for VEGFR-2 and/or VEGFR-3 binding, stimulatory, and inhibitory activities, using *in vitro* and *in vivo* activity assays as described elsewhere herein. To form another VEGF analog of the invention, a VEGF^{+cys} mutant is altered to remove a conserved cysteine corresponding to cys₇₇ of the VEGF165 precursor. Elimination of this cysteine from the VEGF L58C would result in a VEGF analog resembling VEGF-CANΔCHisC156S. This VEGF analog is screened for its

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VEGF-inhibitory activities with respect to VEGFR-2 and/or VEGFR-1 and for VEGF-C like stimulatory or inhibitory activities.

Another noteworthy structural difference between VEGF and VEGF-C is the absence in VEGF-C of several basic residues found in VEGF (e.g., residues Arg₁₀₈, 5 Lys₁₁₀ and His₁₁₂ in the VEGF₁₆₅ precursor shown in Fig. 2) that have been implicated in VEGF receptor binding. See Keyt *et al.*, *J. Biol. Chem.*, 271(10):5638-46 (1996). In another embodiment of the invention, codons for basic residues (lys, arg, his) are substituted into the VEGF-C coding sequence at one or more analogous positions by site-directed mutagenesis. For example, in a preferred embodiment, Glu₁₈₇, Thr₁₈₉, and Pro₁₉₁ 10 in VEGF-C (SEQ ID NO: 8) are replaced with Arg, Lys, and His residues, respectively. The resultant VEGF-C analogs (collectively termed "VEGF-C^{basic}" polypeptides) are recombinantly expressed and screened for VEGFR-1, VEGFR-2, and VEGFR-3 stimulatory and inhibitory activity. The foregoing VEGF and VEGF-C analogs that have VEGF-like activity, VEGF-C-like activity, or that act as inhibitors of VEGF or VEGF-C, 15 are contemplated as additional aspects of the invention. Polynucleotides encoding the analogs also are intended as aspects of the invention.

EXAMPLE 39

EFFECTS OF VEGF-C ON GROWTH AND DIFFERENTIATION OF HUMAN CD34+ PROGENITOR CELLS *IN VITRO*

20 Human CD34+ progenitor cells (HPC, 10×10^3) were isolated from bone marrow or cord blood mononuclear cells using the MACS CD34 Progenitor cell Isolation Kit (Miltenyi Biotec, Bergish Gladbach, Germany), according to the instructions of the manufacturer and cultured in RPMI 1640 medium supplemented with L-glutamine (2.5 mM), penicillin (125 IE/ml), streptomycin (125 µg/ml) and pooled 10 % umbilical cord 25 blood (CB) plasma at 37 °C in a humidified atmosphere in the presence of 5% CO₂ for seven days, with or without VEGF-C and with or without one of the combinations of growth factors described below. Each experiment was performed in triplicate. After seven days, total cell number was evaluated in each culture.

In a first set of experiments, VEGF-C was added, at concentrations ranging 30 from 10 ng/ml to 1 µg/ml, to the cultures of CB CD34+ HPCs. Cell numbers were evaluated at day 7 of culture. When added as a single factor, 100 ng/ml of VEGF-C was

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found support the survival and proliferation of only a few CD34+ HPCs under serum-free conditions. With medium alone, most of the cells died within a culture period of 7 days. However, there were consistently more cells in the cultures provided with the VEGF-C.

A subsequent set of experiments investigated the co-stimulatory effect of VEGF-C in cultures either supplemented with recombinant human stem cell factor (rhSCF, 20 ng/ml PreproTech, Rocky Hill, NY) alone or a combination of granulocyte macrophage colony stimulating factor (rhGM-CSF, 100 ng/ml, Sandoz, Basel, Switzerland) plus SCF. Addition of VEGF-C to SCF-supplemented cultures resulted in a slight co-stimulatory effect on cell growth of CD34+ cells, and this effect was already observable at a VEGF-C concentration of 10 ng/ml. Addition of VEGF-C to GM-CSF- plus SCF-supplemented cultures clearly increased cell yields after 7 days of culture, with an optimum VEGF-C concentration of 100 ng/ml. Additional experiments were conducted to analyze the co-stimulatory effects of 100 ng/ml VEGF-C on total cell yields of serum-free cultures of CB CD34+ HPC cells supplemented with either GM-CSF alone, IL-3 (rhIL-3, 100 U/ml, Behring AG, Marburg, Germany) alone; or a combination of GM-CSF plus IL-3. The results are shown below in the following table:

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Total cell number ($E \times 10^{-3}$) after a culture period of 7 days in RPMI + 10% CBPL, + specified growth factors with (+) or without (-) VEGF-C. (Cell number at day 0 = 10)			
Growth Factor(s)	experiment number	- VEGF-C	+ VEGF-C
GM-CSF	1	11	15
	2	10	17
	3	19	25
	mean \pm SE	13.3 \pm 2.8	19.0 \pm 3.1*
IL-3	1	113	130
	2	107	113
	3	200	433
	4	45	90
	mean \pm SE	116.2 \pm 31.9	191.5 \pm 80.9
GM-CSF + IL-3	1	150	160
	2	130	140
	3	140	155
	mean \pm SE	140.0 \pm 5.7	151.7 \pm 6.0*
GM-CSF + SCF	1	31	37
	2	60	227
	3	47	50
	mean \pm SE	46.0 \pm 8.3	104.7 \pm 61.3

*mean \pm SE; p=0.02

As depicted in the table, VEGF-C led to a consistent enhancement of cell growth when added as a supplement to each growth factor or combination of growth factors tested.

Effect of VEGF-C on granulomonocytic differentiation of CD34+ progenitors

Using cells from the (7 day) plasma-supplemented cultures described above, immunofluorescence triple stainings were performed to analyze the expression of the early granulomonocytic marker molecules lysozyme (LZ) and myeloperoxidase (MPO) as well as the lipopolysaccharide (LPS) receptor CD14. The table below depicts the percentages and numbers of cells expressing MPO and/or LZ:

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Percentages and numbers of cells expressing the markers MPO and LZ after 7 days of culture with (+) or without (-) VEGF-C and specified growth factors						
factor	marker	exp. no.	percent of cells positive for cell marker		numbers of cells positive for cell marker ($E \times 10^3$)	
			- VEGF-C	+ VEGF-C	- VEGF-C	+ VEGF-C
GM-CSF	MPO	1	57	69	6	11
		2	45	53	5	9
		3	18	24	10	13
		mean \pm SE	40.0 \pm 11.0	49.0 \pm 13*	7.0 \pm 1.5	11.0 \pm 1.5*
	LZ	1	54	70	6	11
		2	16	16	2	3
		3	15	23	9	13
		mean \pm SE	28.0 \pm 12.8	36.0 \pm 16.7	5.7 \pm 2.0	9.0 \pm 3.0
5 IL-3	MPO	1	20	28	23	36
		2	37	42	39	48
		3	5	9	10	35
		mean \pm SE	21.0 \pm 9.0	26.0 \pm 9.0	24.0 \pm 8.3	39.7 \pm 4.2
	LZ	1	15	22	17	29
		2	3	3	3	3
		3	3	5	6	22
		mean \pm SE	7.0 \pm 4.0	10.3 \pm 5.8	8.7 \pm 4.0	18.0 \pm 7.0
GM-CSF + IL-3	MPO	1	29	37	46	56
		2	38	40	49	56
		3	6	10	3	6
		mean \pm SE	24.0 \pm 9.0	39.3 \pm 16.6	32.7 \pm 14.8	39.3 \pm 16.6
	LZ	1	18	20	29	30
		2	2	3	3	3
		3	1	2	1	2
		mean \pm SE	7.0 \pm 5.5	8.3 \pm 5.8	11.0 \pm 9.0	12.0 \pm 9.0
10 GM-CSF + SCF	MPO	1	50	51	15	19
		2	16	21	10	48
		mean \pm SE	33.0 \pm 17.0	36.0 \pm 15.0	12.5 \pm 2.5	33.5 \pm 14.5
	LZ	1	15	15	5	6
		2	9	20	5	45
		mean \pm SE	12.0 \pm 3.0	18.0 \pm 2.0	5.0 \pm 0.0	25.5 \pm 19.5

Among the granulomonocytic markers tested, VEGF-C led to an increase in the proportion of LZ+ cells under all culture conditions. In comparison, LZ+CD14+ cells, which represent differentiated monocytic cells only very slightly increased upon addition of VEGF-C (data not shown). Co-stimulation of the cells with VEGF-C increased the expression of MPO, an early granulocytic marker molecule, only modestly, except in combination with both GM-CSF and IL-3, where the increase in the proportion of MPO+ cells was more pronounced.

VEGF-C exerts co-stimulatory effects in combination with M-CSF

In another series of experiments, CD34+ cells were cultured in medium supplemented with 50 ng/ml M-CSF, with or without 100 ng/ml VEGF-C, for seven days. Culture of CD34+ cells in the presence of M-CSF leads to the generation of CD14+ monocytes within 7 days. After seven days, the cultures were analyzed to determine the percentages of CD14+ cells and mean fluorescence intensity. The results are summarized in the table below:

Percentages of CD14 ⁺ cells and mean fluorescence intensity (MFI) of cells cultured with M-CSF in the absence or in the presence of VEGF-C				
	M-CSF alone		M-CSF + VEGF-C	
exp no	% CD14+	MFI	% CD14+	MFI
1	37	20	47	40
2	42	44	54	74
3	32	6	36	7
mean±SE	36.8±2.9	23.3±11.1	45.7±5.2	40.3±19.3

As shown in the table, addition of VEGF-C to these cultures increased both the proportion of CD14+ cells (37% CD14+ cells vs. 46%) and the fluorescence intensity of CD14 expression (MFI 23.3 vs. 40.3). However, cell numbers did not increase upon addition of VEGF-C to M-CSF supplemented cultures. Thus, VEGF-C had a small effect on the differentiation of monocytic cells, but not on their growth.

In the foregoing experiments the presence of VEGF-C was associated with enhanced numbers of cells in cultures of cord blood CD34+ cells. Under all conditions tested (GM-CSF, IL-3, GM-CSF + IL-3; GM-CSF + SCF), co-culture with VEGF-C led

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to an enhancement of proportions of myeloid cells. These results indicate an application for VEGF-C in the stimulation and/or differentiation of CD34+ progenitor cells *in vitro* or *in vivo*. Furthermore, the use of VEGF-C alone also slightly increased the number of surviving cells. The results thus indicate uses for compositions comprising VEGF-C
5 prepared in admixture with the aforementioned or other growth factors, such as VEGF-C, and unit dose formulations comprising VEGF-C packaged together with the aforementioned or other growth factors. Such compositions, unit dose formulations, and methods of their use are intended as further aspects of the present invention.

Deposit of Biological Materials: Plasmid FLT4-L has been deposited
10 with the American Type Culture Collection (ATCC), 12301 Parklawn Dr., Rockville MD 20952 (USA), pursuant to the provisions of the Budapest Treaty, and has been assigned a deposit date of 24 July 1995 and ATCC accession number 97231.

While the present invention has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those in the
15 art. Accordingly, only such limitations as appear in the appended claims should be placed on the invention.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Ludwig Institute for Cancer Research
Helsinki University Licensing
Alitalo, Kari (U.S. only)
Joukov, Vladimir (U.S. only)
- (ii) TITLE OF INVENTION: Vascular Endothelial Growth Factor C (VEGF-C)
Protein and Gene, Mutants Thereof, and Uses Thereof
- (iii) NUMBER OF SEQUENCES: 59
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
 - (B) STREET: 6300 Sears Tower, 233 South Wacker Drive
 - (C) CITY: Chicago
 - (D) STATE: Illinois
 - (E) COUNTRY: United States of America
 - (F) ZIP: 60606-6402
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/795,430
 - (B) FILING DATE: 05-FEB-1997
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: PCT/FI96/00427
 - (B) FILING DATE: 01-AUG-1996
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/671,573
 - (B) FILING DATE: 28-JUN-1996
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/601,132
 - (B) FILING DATE: 14-FEB-1996
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/585,895
 - (B) FILING DATE: 12-JAN-1996
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/510,133
 - (B) FILING DATE: 01-AUG-1995

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(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: 08/340,011
(B) FILING DATE: 14-NOV-1994

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Gass, David A.
(B) REGISTRATION NUMBER: 38,153
(C) REFERENCE/DOCKET NUMBER: 28967/34140

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 312/474-6300
(B) TELEFAX: 312/474-0448
(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4416 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCACGCGCAG CGGCCGGAGA TGCAGCGGGG CGCCGCGCTG TGCCTGCGAC TGTGGCTCTG	60
CCTGGGACTC CTGGACGGCC TGGTGAGTGG CTACTCCATG ACCCCCCCGA CCTTGAACAT	120
CACGGAGGAG TCACACGTCA TCGACACCGG TGACAGCCTG TCCATCTCCT GCAGGGGACA	180
GCACCCCTC GAGTGGGCTT GGCCAGGAGC TCAGGAGGCG CCAGCCACCG GAGACAAGGA	240
CAGCGAGGAC ACGGGGGTGG TGCAGAGCTG CGAGGGCACA GACGCCAGGC CCTACTGCAA	300
GGTGTGCTG CTGCACGAGG TACATGCCAA CGACACAGGC AGCTACGTCT GCTACTACAA	360
GTACATCAAG GCACGCATCG AGGGCACCAC GGCCGCCAGC TCCTACGTGT TCGTGAGAGA	420
CTTTGAGCAG CCATTTCATCA ACAAGCCTGA CACGCTCTTG GTCAACAGGA AGGACGCCAT	480
GTGGGTGCCC TGTCTGGTGT CCATCCCCGG CCTCAATGTC ACGCTGCGCT CGCAAAGCTC	540
GGTGCTGTGG CCAGACGGGC AGGAGGTGGT GTGGGATGAC CGGCGGGGCA TGCTCGTGTC	600
CACGCCACTG CTGCACGATG CCCTGTACCT GCAGTGCGAG ACCACCTGGG GAGACCAGGA	660
CTTCCTTTCC AACCCTTCC TGGTGACAT CACAGGCAAC GAGCTCTATG ACATCCAGCT	720
GTTGCCCAGG AAGTCGCTGG AGCTGCTGGT AGGGGAGAAG CTGGTCCTGA ACTGCACCGT	780
GTGGGCTGAG TTAACTCAG GTGTCACCTT TGACTGGGAC TACCCAGGGA AGCAGGCAGA	840

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GCGGGGTAAG	TGGGTGCCCCG	AGCGACGCTC	CCAGCAGACC	CACACAGAAC	TCTCCAGCAT	900
CCTGACCATC	CACAACGTCA	GCCAGCACGA	CCTGGGCTCG	TATGTGTGCA	AGGCCAACAA	960
CGGCATCCAG	CGATTTTCGGG	AGAGCACCGA	GGTCATTGTG	CATGAAAATC	CCTTCATCAG	1020
CGTCGAGTGG	CTCAAAGGAC	CCATCCTGGA	GGCCACGGCA	GGAGACGAGC	TGGTGAAGCT	1080
GCCCCGTGAAG	CTGGCAGCGT	ACCCCCCGCC	CGAGTTCCAG	TGGTACAAGG	ATGGAAAGGC	1140
ACTGTCCGGG	CGCCACAGTC	CACATGCCCT	GGTGCTCAAG	GAGGTGACAG	AGGCCAGCAC	1200
AGGCACCTAC	ACCCTCGCCC	TGTGGAACTC	CGCTGCTGGC	CTGAGGCGCA	ACATCAGCCT	1260
GGAGCTGGTG	GTGAATGTGC	CCCCCCAGAT	ACATGAGAAG	GAGGCCTCCT	CCCCCAGCAT	1320
CTACTCGCGT	CACAGCCGCC	AGGCCCTCAC	CTGCACGGCC	TACGGGGTGC	CCCTGCCTCT	1380
CAGCATCCAG	TGGCACTGGC	GGCCCTGGAC	ACCCTGCAAG	ATGTTTGCCC	AGCGTAGTCT	1440
CCGGCGGCGG	CAGCAGCAAG	ACCTCATGCC	ACAGTGCCGT	GACTGGAGGG	CGGTGACCAC	1500
GCAGGATGCC	GTGAACCCCA	TCGAGAGCCT	GGACACCTGG	ACCGAGTTTG	TGGAGGGAAA	1560
GAATAAGACT	GTGAGCAAGC	TGGTGATCCA	GAATGCCAAC	GTGTCTGCCA	TGTACAAGTG	1620
TGTGGTCTCC	AACAAGGTGG	GCCAGGATGA	GCGGCTCATC	TACTTCTATG	TGACCACCAT	1680
CCCCGACGGC	TTCACCATCG	AATCCAAGCC	ATCCGAGGAG	CTACTAGAGG	GCCAGCCGGT	1740
GCTCCTGAGC	TGCCAAGCCG	ACAGCTACAA	GTACGAGCAT	CTGCGCTGGT	ACCGCCTCAA	1800
CCTGTCCACG	CTGCACGATG	CGCACGGGAA	CCCCTTCTG	CTCGACTGCA	AGAACGTGCA	1860
TCTGTTTCGCC	ACCCCTCTGG	CCGCCAGCCT	GGAGGAGGTG	GCACCTGGGG	CGCGCCACGC	1920
CACGCTCAGC	CTGAGTATCC	CCCGCGTCGC	GCCCGAGCAC	GAGGGCCACT	ATGTGTGCGA	1980
AGTGCAAGAC	CGGCGCAGCC	ATGACAAGCA	CTGCCACAAG	AAGTACCTGT	CGGTGCAGGC	2040
CCTGGAAGCC	CCTCGGCTCA	CGCAGAACTT	GACCGACCTC	CTGGTGAACG	TGAGCGACTC	2100
GCTGGAGATG	CAGTGCTTGG	TGGCCGGAGC	GCACGCGCCC	AGCATCGTGT	GGTACAAAGA	2160
CGAGAGGCTG	CTGGAGGAAA	AGTCTGGAGT	CGACTTGCGG	GACTCCAACC	AGAAGCTGAG	2220
CATCCAGCGC	GTGCGCGAGG	AGGATGCGGG	ACGCTATCTG	TGCAGCGTGT	GCAACGCCAA	2280
GGGCTGCGTC	AACTCCTCCG	CCAGCGTGCC	CGTGGAAGGC	TCCGAGGATA	AGGGCAGCAT	2340
GGAGATCGTG	ATCCTTGTCG	GTACCGGCGT	CATCGCTGTC	TTCTTCTGGG	TCCTCCTCCT	2400
CCTCATCTTC	TGTAACATGA	GGAGGCCGGC	CCACGCAGAC	ATCAAGACGG	GCTACCTGTC	2460
CATCATCATG	GACCCCGGGG	AGGTGCCTCT	GGAGGAGCAA	TGCGAATACC	TGTCCTACGA	2520
TGCCAGCCAG	TGGGAATTCC	CCCGAGAGCG	GCTGCACCTG	GGGAGAGTGC	TCGGCTACGG	2580

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CGCCTTCGGG	AAGGTGGTGG	AAGCCTCCGC	TTTCGGCATC	CACAAGGGCA	GCAGCTGTGA	2640
CACCGTGGCC	GTGAAAATGC	TGAAAGAGGG	CGCCACGGCC	AGCGAGCACC	GCGCGCTGAT	2700
GTCGGAGCTC	AAGATCCTCA	TTCACATCGG	CAACCACCTC	AACGTGGTCA	ACCTCCTCGG	2760
GGCGTGCACC	AAGCCGCAGG	GCCCCCTCAT	GGTGATCGTG	GAGTTCTGCA	AGTACGGCAA	2820
CCTCTCCAAC	TTCCTGCGCG	CCAAGCGGGA	CGCCTTCAGC	CCCTGCGCGG	AGAAGTCTCC	2880
CGAGCAGCGC	GGACGCTTCC	GCGCCATGGT	GGAGCTCGCC	AGGCTGGATC	GGAGGCGGCC	2940
GGGGAGCAGC	GACAGGGTCC	TCTTCGCGCG	GTTCTCGAAG	ACCGAGGGCG	GAGCGAGGCG	3000
GGCTTCTCCA	GACCAAGAAG	CTGAGGACCT	GTGGCTGAGC	CCGCTGACCA	TGGAAGATCT	3060
TGTCTGCTAC	AGCTTCCAGG	TGGCCAGAGG	GATGGAGTTC	CTGGCTTCCC	GAAAGTGCAT	3120
CCACAGAGAC	CTGGCTGCTC	GGAACATTCT	GCTGTGCGAA	AGCGACGTGG	TGAAGATCTG	3180
TGACTTTTGGC	CTTGCCCCGGG	ACATCTACAA	AGACCCTGAC	TACGTCCGCA	AGGGCAGTGC	3240
CCGGCTGCCC	CTGAAGTGGA	TGGCCCCCTGA	AAGCATCTTC	GACAAGGTGT	ACACCACGCA	3300
GAGTGACGTG	TGGTCCTTTG	GGGTGCTTCT	CTGGGAGATC	TTCTCTCTGG	GGGCCTCCCC	3360
GTACCCTGGG	GTGCAGATCA	ATGAGGAGTT	CTGCCAGCGG	CTGAGAGACG	GCACAAGGAT	3420
GAGGGCCCCG	GAGCTGGCCA	CTCCCGCCAT	ACGCCGCATC	ATGCTGAACT	GCTGGTCCGG	3480
AGACCCCAAG	GCGAGACCTG	CATTCTCGGA	GCTGGTGGAG	ATCCTGGGGG	ACCTGCTCCA	3540
GGGCAGGGGC	CTGCAAGAGG	AAGAGGAGGT	CTGCATGGCC	CCGCGCAGCT	CTCAGAGCTC	3600
AGAAGAGGGC	AGCTTCTCGC	AGGTGTCCAC	CATGGCCCTA	CACATCGCCC	AGGCTGACGC	3660
TGAGGACAGC	CCGCCAAGCC	TGCAGCGCCA	CAGCCTGGCC	GCCAGGTATT	ACAACCTGGGT	3720
GTCCTTTTCCC	GGGTGCCTGG	CCAGAGGGGC	TGAGACCCGT	GGTTCTCTCA	GGATGAAGAC	3780
ATTTGAGGAA	TTCCCCATGA	CCCCAACGAC	CTACAAAGGC	TCTGTGGACA	ACCAGACAGA	3840
CAGTGGGATG	GTGCTGGCCT	CGGAGGAGTT	TGAGCAGATA	GAGAGCAGGC	ATAGACAAGA	3900
AAGCGGCTTC	AGGTAGCTGA	AGCAGAGAGA	GAGAAGGCAG	CATACGTCAG	CATTTTCTTC	3960
TCTGCACTTA	TAAGAAAGAT	CAAAGACTTT	AAGACTTTTCG	CTATTTCTTC	TACTGCTATC	4020
TACTACAAAC	TTCAAAGAGG	AACCAGGAGG	ACAAGAGGAG	CATGAAAGTG	GACAAGGAGT	4080
GTGACCACTG	AAGCACCACA	GGGAAGGGGT	TAGGCCTCCG	GATGACTGCG	GGCAGGCCTG	4140
GATAATATCC	AGCCTCCAC	AAGAAGCTGG	TGGAGCAGAG	TGTTCCCTGA	CTCCTCCAAG	4200
GAAAGGGAGA	CGCCCTTTCA	TGGTCTGCTG	AGTAACAGGT	GCNTTCCCAG	AACTGGCGT	4260
TACTGCTTGA	CCAAAGAGCC	CTCAAGCGGC	CCTTATGCCA	GCGTGACAGA	GGGCTCACCT	4320

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CTTGCCCTTCT AGGTCACTTC TCACACAATG TCCCTTCAGC ACCTGACCCT GTGCCCCGCA 4380
GTTATTCCTT GGTAATATGA GTAATACATC AAAGAG 4416

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 216 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAAGAAAGCG GCTTCAGCTG TAAAGGACCT GGCCAGAATG TGGCTGTGAC CAGGGCACAC 60
CCTGACTCCC AAGGGAGGCG GCGGCGGCCT GAGCGGGGGG CCCGAGGAGG CCAGGTGTTT 120
TACAACAGCG AGTATGGGGA GCTGTCCGAG CCAAGCGAGG AGGACCACTG CTCCCCGTCT 180
GCCCCGCTGA CTTTCTTCAC AGACAACAGC TACTAA 216

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4273 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AAGCTTATCG ATTTCTGAACC CGGGGGTACC GAATTCCTCG AGTCTAGAGG AGCATGCCTG 60
CAGGTCGACC GGGCTCGATC CCCTCGCGAG TTGGTTCAGC TGCTGCCTGA GGCTGGACGA 120
CCTCGCGGAG TTCTACCGGC AGTGCAAATC CGTCGGCATC CAGGAAACCA GCAGCGGCTA 180
TCCGCGCATC CATGCCCCCG AACTGCAGGA GTGGGGAGGC ACGATGGCCG CTTTGGTCCC 240
GGATCTTTGT GAAGGAACCT TACTTCTGTG GTGTGACATA ATTGGACAAA CTACCTACAG 300
AGATTTAAAG CTCTAAGGTA AATATAAAAT TTTTAAGTGT ATAATGTGTT AAACCTACTGA 360
TTCTAATTGT TTGTGTATTT TAGATTCCAA CCTATGGAAC TGATGAATGG GAGCAGTGGT 420
GGAATGCCTT TAATGAGGAA AACCTGTTTT GCTCAGAAGA AATGCCATCT AGTGATGATG 480

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AGGCTACTGC	TGACTCTCAA	CATTCTACTC	CTCCAAAAAA	GAAGAGAAAG	GTAGAAGACC	540
CCAAGGACTT	TCCTTCAGAA	TTGCTAAGTT	TTTTGAGTCA	TGCTGTGTTT	AGTAATAGAA	600
CTCTTGCTTG	CTTTGCTATT	TACACCACAA	AGGAAAAAGC	TGCACTGCTA	TACAAGAAAA	660
TTATGGAAAA	ATATTCTGTA	ACCTTTTATAA	GTAGGCATAA	CAGTTATAAT	CATAACATAC	720
TGTTTTTTTCT	TACTCCACAC	AGGCATAGAG	TGTCTGCTAT	TAATAACTAT	GCTCAAAAAT	780
TGTGTACCTT	TAGCTTTTTTA	ATTTGTAAAG	GGGTAAATAA	GGAATATTTG	ATGTATAGTG	840
CCTTGACTAG	AGATCATAAT	CAGCCATACC	ACATTTGTAG	AGGTTTTACT	TGCTTTAAAA	900
AACCTCCCAC	ACCTCCCCCT	GAACCTGAAA	CATAAAATGA	ATGCAATTGT	TGTTGTAAAC	960
TTGTTTATTG	CAGCTTATAA	TGGTTACAAA	TAAAGCAATA	GCATCACAAA	TTTCACAAAT	1020
AAAGCATTTT	TTTCACTGCA	TTCTAGTTGT	GGTTTGTCCA	AACTCATCAA	TGTATCTTAT	1080
CATGTCTGGA	TCTGCCGGTC	TCCCTATAGT	GAGTCGTATT	AATTTGATA	AGCCAGGTTA	1140
ACCTGCATTA	ATGAATCGGC	CAACGCGCGG	GGAGAGGCGG	TTTGCGTATT	GGGCGCTCTT	1200
CCGCTTCCTC	GCTCACTGAC	TCGCTGCGCT	CGGTCGTTCC	GCTGCGGCGA	GCGGTATCAG	1260
CTCACTCAAA	GGCGGTAATA	CGGTTATCCA	CAGAATCAGG	GGATAACGCA	GGAAAGAACA	1320
TGTGAGCAAA	AGGCCAGCAA	AAGGCCAGGA	ACCGTAAAAA	GGACGCGTTG	CTGGCGTTTT	1380
TCCATAGGCT	CCGCCCCCCT	GACGAGCATC	ACAAAAATCG	ACGCTCAAGT	CAGAGGTGGC	1440
GAAACCCGAC	AGGACTATAA	AGATACCAGG	CGTTTCCCCC	TGGAAGCTCC	CTCGTGCGCT	1500
CTCCTGTTCC	GACCCTGCCG	CTTACCGGAT	ACCTGTCCGC	CTTTCTCCCT	TCGGGAAGCG	1560
TGGCGCTTTC	TCAATGCTCA	CGCTGTAGGT	ATCTCAGTTC	GGTGTAGGTC	GTTGCTCCCA	1620
AGCTGGGCTG	TGTGCACGAA	CCCCCGTTT	AGCCCGACCG	CTGCGCCTTA	TCCGGTAACT	1680
ATCGTCTTGA	GTCCAACCCG	GTAAGACACG	ACTTATCGCC	ACTGGCAGCA	GCCACTGGTA	1740
ACAGGATTAG	CAGAGCGAGG	TATGTAGGCG	GTGCTACAGA	GTTCTTGAAG	TGGTGGCCTA	1800
ACTACGGCTA	CACTAGAAGG	ACAGTATTTG	GTATCTGCGC	TCTGCTGAAG	CCAGTTACCT	1860
TCGGAAAAAG	AGTTGGTAGC	TCTTGATCCG	GCAAACAAAC	CACCGCTGGT	AGCGGTGGTT	1920
TTTTTGTTTG	CAAGCAGCAG	ATTACGCGCA	GAAAAAAAGG	ATCTCAAGAA	GATCCTTTGA	1980
TCTTTTCTAC	GGGGTCTGAC	GCTCAGTGGA	ACGAAAACTC	ACGTAAAGGG	ATTTTGGTCA	2040
TGAGATTATC	AAAAAGGATC	TTCACCTAGA	TCCTTTTAAA	TTAAAAATGA	AGTTTTAAAT	2100
CAATCTAAAG	TATATATGAG	TAAACTTGGT	CTGACAGTTA	CCAATGCTTA	ATCAGTGAGG	2160
CACCTATCTC	AGCGATCTGT	CTATTTCTGT	CATCCATAGT	TGCCTGACTC	CCCGTCGTGT	2220

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AGATAACTAC	GATACGGGAG	GGCTTACCAT	CTGGCCCCAG	TGCTGCAATG	ATACCGCGAG	2280
ACCCACGCTC	ACCGGCTCCA	GATTTATCAG	CAATAAACCA	GCCAGCCGGA	AGGGCCGAGC	2340
GCAGAAGTGG	TCCTGCAACT	TTATCCGCCT	CCATCCAGTC	TATTAATTGT	TGCCGGGAAG	2400
CTAGAGTAAG	TAGTTCGCCA	GTTAATAGTT	TGCGCAACGT	TGTTGCCATT	GCTACAGGCA	2460
TCGTGGTGTC	ACGCTCGTCG	TTTGGTATGG	CTTCATTCAG	CTCCGGTTCC	CAACGATCAA	2520
GGCGAGTTAC	ATGATCCCCC	ATGTTGTGCA	AAAAAGCGGT	TAGCTCCTTC	GGTCCTCCGA	2580
TCGTTGTCAG	AAGTAAGTTG	GCCGCAGTGT	TATCACTCAT	GGTTATGGCA	GCACTGCATA	2640
ATTCTCTTAC	TGTCATGCCA	TCCGTAAGAT	GCTTTTCTGT	GACTGGTGAG	TACTCAACCA	2700
AGTCATTCTG	AGAATAGTGT	ATGCGGCGAC	CGAGTTGCTC	TTGCCCCGGC	TCAATACGGG	2760
ATAATACCGC	GCCACATAGC	AGAACTTTAA	AAGTGCTCAT	CATTGGAAAA	CGTTCTTCGG	2820
GGCGAAAACT	CTCAAGGATC	TTACCGCTGT	TGAGATCCAG	TTCGATGTAA	CCCACTCGTG	2880
CACCCAACCTG	ATCTTCAGCA	TCTTTTACTT	TCACCAGCGT	TTCTGGGTGA	GCAAAAACAG	2940
GAAGGCAAAA	TGCCGCAAAA	AAGGGAATAA	GGGCGACACG	GAAATGTTGA	ATACTCATAC	3000
TCTTCCTTTT	TCAATATTAT	TGAAGCATTT	ATCAGGGTTA	TTGTCTCATG	AGCGGATACA	3060
TATTTGAATG	TATTTAGAAA	AATAAACAAA	TAGGGGTTCC	GCGCACATTT	CCCCGAAAAG	3120
TGCCACCTGA	CGTCTAAGAA	ACCATTATTA	TCATGACATT	AACCTATAAA	AATAGGCGTA	3180
TCACGAGGCC	CTTTCGTCTC	GCGCGTTTCG	GTGATGACGG	TGAAAACCTC	TGACACATGC	3240
AGCTCCCGGA	GACGGTCACA	GCTTGTCGTG	AAGCGGATGC	CGGGAGCAGA	CAAGCCCCTC	3300
AGGGCGCGTC	AGCGGGTGTT	GGCGGGTGTC	GGGGCTGGCT	TAACATATGC	GCATCAGAGC	3360
AGATTGTACT	GAGAGTGCAC	CATATGGACA	TATTGTCTGT	AGAACGCGGC	TACAATTAAT	3420
ACATAACCTT	ATGTATCATA	CACATACGAT	TTAGGTGACA	CTATAGAACT	CGAGCAGAGC	3480
TTCCAAATTG	AGAGAGAGGC	TTAATCAGAG	ACAGAACTG	TTTGAGTCAA	CTCAAGGATG	3540
GTTTGAGGGG	CTGTTTAACA	GATCCCCCTG	GTTTACCACC	TTGATATCTA	CCATTATGGG	3600
ACCCCTCATT	GTACTCCTAA	TGATTTTGCT	CTTCGGACCC	TGCATTCTTA	ATCGATTAGT	3660
CCAATTTGTT	AAAGACAGGA	TATCAGTGGT	CCAGGCTCTA	GTTTTGACTC	AACAATATCA	3720
CCAGCTGAAG	CCTATAGAGT	ACGAGCCATA	GATAAAATAA	AAGATTTTAT	TTAGTCTCCA	3780
GAAAAAGGGG	GGAATGAAAG	ACCCACCTG	TAGGTTTGGC	AAGCTAGCTT	AAGTAACGCC	3840
ATTTTGCAAG	GCATGGAAAA	ATACATAACT	GAGAATAGAG	AAGTTCAGAT	CAAGGTCAGG	3900
AACAGATGGA	ACAGCTGAAT	ATGGGCCAAA	CAGGATATCT	GTGGTAAGCA	GTTCCTGCCC	3960

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CGGCTCAGGG CCAAGAACAG ATGGAACAGC TGAATATGGG CCAAACAGGA TATCTGTGGT 4020
 AAGCAGTTCC TGCCCCGGCT CAGGGCCAAG AACAGATGGT CCCCAGATGC GGTCCAGCCC 4080
 TCAGCAGTTT CTAGAGAACC ATCAGATGTT TCCAGGGTGC CCCAAGGACC TGAAATGACC 4140
 CTGTGCCTTA TTTGAACTAA CCAATCAGTT CGCTTCTCGC TTCTGTTTCGC GCGCTTCTGC 4200
 TCCCCGAGCT CAATAAAAGA GCCCACAACC CCTCACTCGG GCGCCAGTC CTCCGATTGA 4260
 CTGAGTCGCC CGG 4273

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp
 1 5 10 15
 Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
 20 25 30
 His Arg Gln Glu Ser Gly Phe Arg
 35 40

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile
 1 5 10 15
 Leu Lys

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 219 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TCACTATAGG GAGACCCAAG CTTGGTACCG AGCTCGGATC CACTAGTAAC GGCCGCCAGT	60
GTGGTGGAA TCGACGAACT CATGACTGTA CTCTACCCAG AATATTGGAA AATGTACAAG	120
TGTCAGCTAA GGCAAGGAGG CTGGCAACAT AACAGAGAAC AGGCCAACCT CAACTCAAGG	180
ACAGAAGAGA CTATAAAATT CGCTGCAGCA CACTACAAC	219

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1997 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCCCCCCCGC CTCTCCAAAA AGCTACACCG ACGCGGACCG CGGCGGCGTC CTCCCTCGCC	60
CTCGCTTCAC CTCGCGGGCT CCGAATGCGG GGAGCTCGGA TGTCCGGTTT CCTGTGAGGC	120
TTTACCTGA CACCCGCCGC CTTTCCCCGG CACTGGCTGG GAGGGCGCCC TGCAAAGTTG	180
GGAACGCGGA GCCCCGGACC CGCTCCCGCC GCCTCCGGCT CGCCAGGGG GGGTCGCCGG	240
GAGGAGCCCCG GGGGAGAGGG ACCAGGAGGG GCCCGCGGCC TCGCAGGGGC GCCCGCGCCC	300
CCACCCCTGC CCCC GCCAGC GGACCGGTCC CCCACCCCGG GTCCTTCCAC C ATG CAC	357
	Met His

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TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG	405
Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Ala Leu	
5 10 15	
CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC TTC GAG TCC	453
Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe Glu Ser	
20 25 30	
GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT	501
Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala	
35 40 45 50	
TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA	549
Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val	
55 60 65	
GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG	597
Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys	
70 75 80	
TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC	645
Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn	
85 90 95	
CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT	693
Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr	
100 105 110	
AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA	741
Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln	
115 120 125 130	
TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC	789
Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val	
135 140 145	
GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT	837
Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys	
150 155 160	
GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG	885
Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr	
165 170 175	
AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA	933
Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln	
180 185 190	
GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA	981
Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg	
195 200 205 210	
TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA	1029
Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg	
215 220 225	

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CGT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC	1077
Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr	
230 235 240	
TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT	1125
Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala	
245 250 255	
CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT	1173
Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp	
260 265 270	
GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC	1221
Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr	
275 280 285 290	
TGT CAG TGT GTC TGC AGA GCG GGG CTT CGG CCT GCC AGC TGT GGA CCC	1269
Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro	
295 300 305	
CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA	1317
His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys	
310 315 320	
CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA	1365
Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr	
325 330 335	
TGC CAG TGT GTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT	1413
Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn	
340 345 350	
CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG	1461
Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu	
355 360 365 370	
TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG	1509
Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg	
375 380 385	
CCA TGT ACG AAC CGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT	1557
Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser	
390 395 400	
GAA GAA GTG TGT CGT TGT GTC CCT TCA TAT TGG AAA AGA CCA CAA ATG	1605
Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met	
405 410 415	
AGC TAAGATTGTA CTGTTTTCCA GTTCATCGAT TTTCTATTAT GGAAACTGT	1658
Ser	
GTTGCCACAG TAGAACTGTC TGTGAACAGA GAGACCCTTG TGGGTCCATG CTAACAAAGA	1718
CAAAAGTCTG TCTTTCCTGA ACCATGTGGA TAACTTTACA GAAATGGACT GGAGCTCATC	1778
TGCAAAAGGC CTCTTGTAAG GACTGGTTTT CTGCCAATGA CCAAACAGCC AAGATTTTCC	1838

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TCTTGTGATT TCTTTAAAAG AATGACTATA TAATTTATTT CCACTAAAAA TATTGTTTCT 1898
 GCATTCATTT TTATAGCAAC AACAAATTGGT AAAACTCACT GTGATCAATA TTTTATATC 1958
 ATGCAAAATA TGTTTAAAAT AAAATGAAAA TTGTATTAT 1997

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 419 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
 1 5 10 15
 Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe
 20 25 30
 Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
 35 40 45
 Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
 50 55 60
 Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
 65 70 75 80
 Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
 85 90 95
 Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
 100 105 110
 His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
 115 120 125
 Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
 130 135 140
 Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
 145 150 155 160
 Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
 165 170 175
 Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
 180 185 190
 Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
 195 200 205

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Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
 210 215 220
 Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
 225 230 235 240
 Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
 245 250 255
 Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
 260 265 270
 Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
 275 280 285
 Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
 290 295 300
 Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
 305 310 315 320
 Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
 325 330 335
 Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
 340 345 350
 Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
 355 360 365
 Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
 370 375 380
 Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
 385 390 395 400
 Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
 405 410 415
 Gln Met Ser

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu
 1 5 10 15

Lys

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1836 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 168..1412

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCGGCCGCGT CGACGCAAAA GTTGCAGGCC GCCGAGTCCC GGGAGACGCT CGCCCAGGGG 60
 GGTCCCCGGG AGGAAACCAC GGGACAGGGA CCAGGAGAGG ACCTCAGCCT CACGCCCCAG 120
 CCTGCGCCAG CCAACGGACC GGCCTCCCTG CTCCCGGTCC ATCCACC ATG CAC TTG 176
 Met His Leu
 1
 CTG TGC TTC TTG TCT CTG GCG TGT TCC CTG CTC GCC GCT GCG CTG ATC 224
 Leu Cys Phe Leu Ser Leu Ala Cys Ser Leu Leu Ala Ala Ala Leu Ile
 5 10 15
 CCC AGT CCG CGC GAG GCG CCC GCC ACC GTC GCC GCC TTC GAG TCG GGA 272
 Pro Ser Pro Arg Glu Ala Pro Ala Thr Val Ala Ala Phe Glu Ser Gly
 20 25 30 35
 CTG GGC TTC TCG GAA GCG GAG CCC GAC GGG GGC GAG GTC AAG GCT TTT 320
 Leu Gly Phe Ser Glu Ala Glu Pro Asp Gly Gly Glu Val Lys Ala Phe
 40 45 50
 GAA GGC AAA GAC CTG GAG GAG CAG TTG CGG TCT GTG TCC AGC GTA GAT 368
 Glu Gly Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val Asp
 55 60 65
 GAG CTG ATG TCT GTC CTG TAC CCA GAC TAC TGG AAA ATG TAC AAG TGC 416
 Glu Leu Met Ser Val Leu Tyr Pro Asp Tyr Trp Lys Met Tyr Lys Cys
 70 75 80
 CAG CTG CGG AAA GGC GGC TGG CAG CAG CCC ACC CTC AAT ACC AGG ACA 464
 Gln Leu Arg Lys Gly Gly Trp Gln Gln Pro Thr Leu Asn Thr Arg Thr
 85 90 95

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GGG GAC AGT GTA AAA TTT GCT GCT GCA CAT TAT AAC ACA GAG ATC CTG Gly Asp Ser Val Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu 100 105 110 115	512
AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGT GAG Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu 120 125 130	560
GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GCA GCC ACA AAC ACC TTC Val Cys Ile Asp Val Gly Lys Glu Phe Gly Ala Ala Thr Asn Thr Phe 135 140 145	608
TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAC Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn 150 155 160	656
AGC GAG GGG CTG CAG TGC ATG AAC ACC AGC ACA GGT TAC CTC AGC AAG Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Gly Tyr Leu Ser Lys 165 170 175	704
ACG TTG TTT GAA ATT ACA GTG CCT CTC TCA CAA GGC CCC AAA CCA GTC Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val 180 185 190 195	752
ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGG TGC ATG TCT AAA CTG Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu 200 205 210	800
GAT GTT TAC AGA CAA GTT CAT TCA ATT ATT AGA CGT TCT CTG CCA GCA Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala 215 220 225	848
ACA TTA CCA CAG TGT CAG GCA GCT AAC AAG ACA TGT CCA ACA AAC TAT Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr 230 235 240	896
GTG TGG AAT AAC TAC ATG TGC CGA TGC CTG GCT CAG CAG GAT TTT ATC Val Trp Asn Asn Tyr Met Cys Arg Cys Leu Ala Gln Gln Asp Phe Ile 245 250 255	944
TTT TAT TCA AAT GTT GAA GAT GAC TCA ACC AAT GGA TTC CAT GAT GTC Phe Tyr Ser Asn Val Glu Asp Asp Ser Thr Asn Gly Phe His Asp Val 260 265 270 275	992
TGT GGA CCC AAC AAG GAG CTG GAT GAA GAC ACC TGT CAG TGT GTC TGC Cys Gly Pro Asn Lys Glu Leu Asp Glu Asp Thr Cys Gln Cys Val Cys 280 285 290	1040
AAG GGG GGG CTT CGG CCA TCT AGT TGT GGA CCC CAC AAA GAA CTA GAT Lys Gly Gly Leu Arg Pro Ser Ser Cys Gly Pro His Lys Glu Leu Asp 295 300 305	1088
AGA GAC TCA TGT CAG TGT GTC TGT AAA AAC AAA CTT TTC CCT AAT TCA Arg Asp Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Asn Ser 310 315 320	1136

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TGT GGA GCC AAC AGG GAA TTT GAT GAG AAT ACA TGT CAG TGT GTA TGT	1184
Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys	
325 330 335	
AAA AGA ACG TGT CCA AGA AAT CAG CCC CTG AAT CCT GGG AAA TGT GCC	1232
Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala	
340 345 350 355	
TGT GAA TGT ACA GAA AAC ACA CAG AAG TGC TTC CTT AAA GGG AAG AAG	1280
Cys Glu Cys Thr Glu Asn Thr Gln Lys Cys Phe Leu Lys Gly Lys Lys	
360 365 370	
TTC CAC CAT CAA ACA TGC AGT TGT TAC AGA AGA CCG TGT GCG AAT CGA	1328
Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Ala Asn Arg	
375 380 385	
CTG AAG CAT TGT GAT CCA GGA CTG TCC TTT AGT GAA GAA GTA TGC CGC	1376
Leu Lys His Cys Asp Pro Gly Leu Ser Phe Ser Glu Glu Val Cys Arg	
390 395 400	
TGT GTC CCA TCG TAT TGG AAA AGG CCA CAT CTG AAC TAAGATCATA	1422
Cys Val Pro Ser Tyr Trp Lys Arg Pro His Leu Asn	
405 410 415	
CCAGTTTTC A GTCAGTCACA GTCATTTACT CTCTTGAAGA CTGTTGGAAC AGCACTTAGC	1482
ACTGTCTATG CACAGAAAGA CTCTGTGGGA CCACATGGTA ACAGAGGCCC AAGTCTGTGT	1542
TTATTGAACC ATGTGGATTA CTGCGGGAGA GGACTGGCAC TCATGTGCAA AAAAAACCTC	1602
TTCAAAGACT GGTTTTCTGC CAGGGACCAG ACAGCTGAGG TTTTCTCTT GTGATTTAAA	1662
AAAAGAATGA CTATATAATT TATTTCCACT AAAAATATTG TTCCTGCATT CATTTTTATA	1722
GCAATAACAA TTGGTAAAGC TCACTGTGAT CAGTATTTTT ATAACATGCA AACTATGTT	1782
TAAAATAAAA TGAAAATTGT ATTATAAAAA AAAAAAAAAA AAAAAAAAAA GCTT	1836

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 415 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met His Leu Leu Cys Phe Leu Ser Leu Ala Cys Ser Leu Leu Ala Ala	
1 5 10 15	
Ala Leu Ile Pro Ser Pro Arg Glu Ala Pro Ala Thr Val Ala Ala Phe	
20 25 30	

Glu	Ser	Gly	Leu	Gly	Phe	Ser	Glu	Ala	Glu	Pro	Asp	Gly	Gly	Glu	Val
		35						40					45		
Lys	Ala	Phe	Glu	Gly	Lys	Asp	Leu	Glu	Glu	Gln	Leu	Arg	Ser	Val	Ser
	50					55					60				
Ser	Val	Asp	Glu	Leu	Met	Ser	Val	Leu	Tyr	Pro	Asp	Tyr	Trp	Lys	Met
65					70					75					80
Tyr	Lys	Cys	Gln	Leu	Arg	Lys	Gly	Gly	Trp	Gln	Gln	Pro	Thr	Leu	Asn
				85					90					95	
Thr	Arg	Thr	Gly	Asp	Ser	Val	Lys	Phe	Ala	Ala	Ala	His	Tyr	Asn	Thr
			100					105					110		
Glu	Ile	Leu	Lys	Ser	Ile	Asp	Asn	Glu	Trp	Arg	Lys	Thr	Gln	Cys	Met
		115					120					125			
Pro	Arg	Glu	Val	Cys	Ile	Asp	Val	Gly	Lys	Glu	Phe	Gly	Ala	Ala	Thr
	130					135					140				
Asn	Thr	Phe	Phe	Lys	Pro	Pro	Cys	Val	Ser	Val	Tyr	Arg	Cys	Gly	Gly
145					150					155					160
Cys	Cys	Asn	Ser	Glu	Gly	Leu	Gln	Cys	Met	Asn	Thr	Ser	Thr	Gly	Tyr
				165					170					175	
Leu	Ser	Lys	Thr	Leu	Phe	Glu	Ile	Thr	Val	Pro	Leu	Ser	Gln	Gly	Pro
			180					185					190		
Lys	Pro	Val	Thr	Ile	Ser	Phe	Ala	Asn	His	Thr	Ser	Cys	Arg	Cys	Met
		195					200					205			
Ser	Lys	Leu	Asp	Val	Tyr	Arg	Gln	Val	His	Ser	Ile	Ile	Arg	Arg	Ser
	210					215					220				
Leu	Pro	Ala	Thr	Leu	Pro	Gln	Cys	Gln	Ala	Ala	Asn	Lys	Thr	Cys	Pro
225					230					235					240
Thr	Asn	Tyr	Val	Trp	Asn	Asn	Tyr	Met	Cys	Arg	Cys	Leu	Ala	Gln	Gln
				245					250					255	
Asp	Phe	Ile	Phe	Tyr	Ser	Asn	Val	Glu	Asp	Asp	Ser	Thr	Asn	Gly	Phe
			260					265					270		
His	Asp	Val	Cys	Gly	Pro	Asn	Lys	Glu	Leu	Asp	Glu	Asp	Thr	Cys	Gln
	275						280					285			
Cys	Val	Cys	Lys	Gly	Gly	Leu	Arg	Pro	Ser	Ser	Cys	Gly	Pro	His	Lys
	290					295					300				
Glu	Leu	Asp	Arg	Asp	Ser	Cys	Gln	Cys	Val	Cys	Lys	Asn	Lys	Leu	Phe
305					310					315					320
Pro	Asn	Ser	Cys	Gly	Ala	Asn	Arg	Glu	Phe	Asp	Glu	Asn	Thr	Cys	Gln
				325					330					335	

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Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly
 340 345 350
 Lys Cys Ala Cys Glu Cys Thr Glu Asn Thr Gln Lys Cys Phe Leu Lys
 355 360 365
 Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys
 370 375 380
 Ala Asn Arg Leu Lys His Cys Asp Pro Gly Leu Ser Phe Ser Glu Glu
 385 390 395 400
 Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro His Leu Asn
 405 410 415

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1741 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 453..1706

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCGGGCGCGG AGCGCTCCGC GCGCAGCCGC CGGGCCGGGC CGGCGCGCGG AGGGCGCGCT 60
 GCGAGCGGCC ACTGGGTCCT GCTTCCCTCC TTCCTCTCCC TCCTCCTCCT CTCCTTCTC 120
 TCTGCGCTTT CCACCGCTCC CGAGCGAGCG CACGCTCGGA TGTCCGGTTT CTTGGTGGGT 180
 TTTTACCTG GCAAAGTCCG GATAACTTCG GTGAGAATTT GCAAAGAGGC TGGGAGCTCC 240
 CCTGCAGGCG TCTGGGAGCT GCTGCCGCCG TCGCATCTTC TCCATCCCGC GGATTTTACT 300
 GCCTTGATA TTGCGAGGGG AGGGAGGGGG GTGAGGACAG CAAAAGAAA GGGGTGGGGG 360
 GGGGGAGAGA AAAGGAAAAG AAGGAGCCTC GGAATTGTGC CCGCATTCCT GCGTGCCCCC 420
 GCGGCCCCCC TCCGCTCTGC CATCTCCGCA CA ATG CAC TTG CTG GAG ATG CTC 473
 Met His Leu Leu Glu Met Leu
 1 5
 TCC CTG GGC TGC TGC CTC GCT GCT GGC GCC GTG CTC CTG GGA CCC CGG 521
 Ser Leu Gly Cys Cys Leu Ala Ala Gly Ala Val Leu Leu Gly Pro Arg
 10 15 20

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CAG CCG CCC GTC GCC GCC GCC TAC GAG TCC GGG CAC GGC TAC TAC GAG Gln Pro Pro Val Ala Ala Ala Tyr Glu Ser Gly His Gly Tyr Tyr Glu 25 30 35	569
GAG GAG CCC GGT GCC GGG GAA CCC AAG GCT CAT GCA AGC AAA GAC CTG Glu Glu Pro Gly Ala Gly Glu Pro Lys Ala His Ala Ser Lys Asp Leu 40 45 50 55	617
GAA GAG CAG TTG CGA TCT GTG TCC AGT GTG GAT GAA CTC ATG ACA GTA Glu Glu Gln Leu Arg Ser Val Ser Ser Val Asp Glu Leu Met Thr Val 60 65 70	665
CTT TAC CCA GAA TAC TGG AAA ATG TTC AAA TGT CAG TTG AGG AAA GGA Leu Tyr Pro Glu Tyr Trp Lys Met Phe Lys Cys Gln Leu Arg Lys Gly 75 80 85	713
GGT TGG CAA CAC AAC AGG GAA CAC TCC AGC TCT GAT ACA AGA TCA GAT Gly Trp Gln His Asn Arg Glu His Ser Ser Ser Asp Thr Arg Ser Asp 90 95 100	761
GAT TCA TTG AAA TTT GCC GCA GCA CAT TAT AAT GCA GAG ATC CTG AAA Asp Ser Leu Lys Phe Ala Ala Ala His Tyr Asn Ala Glu Ile Leu Lys 105 110 115	809
AGT ATT GAT ACT GAA TGG AGA AAA ACC CAG GGC ATG CCA CGT GAA GTG Ser Ile Asp Thr Glu Trp Arg Lys Thr Gln Gly Met Pro Arg Glu Val 120 125 130 135	857
TGT GTG GAT TTG GGG AAA GAG TTT GGA GCA ACT ACA AAC ACC TTC TTT Cys Val Asp Leu Gly Lys Glu Phe Gly Ala Thr Thr Asn Thr Phe Phe 140 145 150	905
AAA CCC CCG TGT GTA TCC ATC TAC AGA TGT GGA GGT TGC TGC AAT AGT Lys Pro Pro Cys Val Ser Ile Tyr Arg Cys Gly Gly Cys Cys Asn Ser 155 160 165	953
GAA GGA CTC CAG TGT ATG AAT ATC AGC ACA AAT TAC ATC AGC AAG ACA Glu Gly Leu Gln Cys Met Asn Ile Ser Thr Asn Tyr Ile Ser Lys Thr 170 175 180	1001
TTG TTT GAG ATT ACA GTG CCT CTC TCT CAT GGC CCC AAA CCT GTA ACA Leu Phe Glu Ile Thr Val Pro Leu Ser His Gly Pro Lys Pro Val Thr 185 190 195	1049
GTC AGT TTT GCC AAT CAC ACG TCC TGC CGA TGC ATG TCT AAG TTG GAT Val Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp 200 205 210 215	1097
GTT TAC AGA CAA GTT CAT TCT ATC ATA AGA CGT TCC TTG CCA GCA ACA Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr 220 225 230	1145
CAA ACT CAG TGT CAT GTG GCA AAC AAG ACC TGT CCA AAA AAT CAT GTC Gln Thr Gln Cys His Val Ala Asn Lys Thr Cys Pro Lys Asn His Val 235 240 245	1193

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TGG AAT AAT CAG ATT TGC AGA TGC TTA GCA CAG CAC GAT TTT GGT TTC	1241
Trp Asn Asn Gln Ile Cys Arg Cys Leu Ala Gln His Asp Phe Gly Phe	
250 255 260	
TCT TCT CAC CTT GGA GAT TCT GAC ACA TCT GAA GGA TTC CAT ATT TGT	1289
Ser Ser His Leu Gly Asp Ser Asp Thr Ser Glu Gly Phe His Ile Cys	
265 270 275	
GGG CCC AAC AAA GAG CTG GAT GAA GAA ACC TGT CAA TGC GTC TGC AAA	1337
Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Lys	
280 285 290 295	
GGA GGT GTG CGG CCC ATA AGC TGT GGC CCT CAC AAA GAA CTA GAC AGG	1385
Gly Gly Val Arg Pro Ile Ser Cys Gly Pro His Lys Glu Leu Asp Arg	
300 305 310	
GCA TCA TGT CAG TGC ATG TGC AAA AAC AAA CTG CTC CCC AGT TCC TGT	1433
Ala Ser Cys Gln Cys Met Cys Lys Asn Lys Leu Leu Pro Ser Ser Cys	
315 320 325	
GGG CCT AAC AAA GAA TTT GAT GAA GAA AAG TGC CAG TGT GTA TGT AAA	1481
Gly Pro Asn Lys Glu Phe Asp Glu Glu Lys Cys Gln Cys Val Cys Lys	
330 335 340	
AAG ACC TGT CCC AAA CAT CAT CCA CTA AAT CCT GCA AAA TGC ATC TGC	1529
Lys Thr Cys Pro Lys His His Pro Leu Asn Pro Ala Lys Cys Ile Cys	
345 350 355	
GAA TGT ACA GAA TCT CCC AAT AAA TGT TTC TTA AAA GGA AAA AGA TTT	1577
Glu Cys Thr Glu Ser Pro Asn Lys Cys Phe Leu Lys Gly Lys Arg Phe	
360 365 370 375	
CAT CAC CAG ACA TGC AGT TGT TAC AGA CCA CCA TGT ACA GTC CGA ACG	1625
His His Gln Thr Cys Ser Cys Tyr Arg Pro Pro Cys Thr Val Arg Thr	
380 385 390	
AAA CGC TGT GAT GCT GGA TTT CTG TTA GCT GAA GAA GTG TGC CGC TGT	1673
Lys Arg Cys Asp Ala Gly Phe Leu Leu Ala Glu Glu Val Cys Arg Cys	
395 400 405	
GTA CGC ACA TCT TGG AAA AGA CCA CTT ATG AAT TAAGCGAAGA AAGCACTACT	1726
Val Arg Thr Ser Trp Lys Arg Pro Leu Met Asn	
410 415	
CGCTATATAG TGTCG	1741

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 418 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met	His	Leu	Leu	Glu	Met	Leu	Ser	Leu	Gly	Cys	Cys	Leu	Ala	Ala	Gly	1	5	10	15
Ala	Val	Leu	Leu	Gly	Pro	Arg	Gln	Pro	Pro	Val	Ala	Ala	Ala	Tyr	Glu	20	25	30	
Ser	Gly	His	Gly	Tyr	Tyr	Glu	Glu	Glu	Pro	Gly	Ala	Gly	Glu	Pro	Lys	35	40	45	
Ala	His	Ala	Ser	Lys	Asp	Leu	Glu	Glu	Gln	Leu	Arg	Ser	Val	Ser	Ser	50	55	60	
Val	Asp	Glu	Leu	Met	Thr	Val	Leu	Tyr	Pro	Glu	Tyr	Trp	Lys	Met	Phe	65	70	75	80
Lys	Cys	Gln	Leu	Arg	Lys	Gly	Gly	Trp	Gln	His	Asn	Arg	Glu	His	Ser	85	90	95	
Ser	Ser	Asp	Thr	Arg	Ser	Asp	Asp	Ser	Leu	Lys	Phe	Ala	Ala	Ala	His	100	105	110	
Tyr	Asn	Ala	Glu	Ile	Leu	Lys	Ser	Ile	Asp	Thr	Glu	Trp	Arg	Lys	Thr	115	120	125	
Gln	Gly	Met	Pro	Arg	Glu	Val	Cys	Val	Asp	Leu	Gly	Lys	Glu	Phe	Gly	130	135	140	
Ala	Thr	Thr	Asn	Thr	Phe	Phe	Lys	Pro	Pro	Cys	Val	Ser	Ile	Tyr	Arg	145	150	155	160
Cys	Gly	Gly	Cys	Cys	Asn	Ser	Glu	Gly	Leu	Gln	Cys	Met	Asn	Ile	Ser	165	170	175	
Thr	Asn	Tyr	Ile	Ser	Lys	Thr	Leu	Phe	Glu	Ile	Thr	Val	Pro	Leu	Ser	180	185	190	
His	Gly	Pro	Lys	Pro	Val	Thr	Val	Ser	Phe	Ala	Asn	His	Thr	Ser	Cys	195	200	205	
Arg	Cys	Met	Ser	Lys	Leu	Asp	Val	Tyr	Arg	Gln	Val	His	Ser	Ile	Ile	210	215	220	
Arg	Arg	Ser	Leu	Pro	Ala	Thr	Gln	Thr	Gln	Cys	His	Val	Ala	Asn	Lys	225	230	235	240
Thr	Cys	Pro	Lys	Asn	His	Val	Trp	Asn	Asn	Gln	Ile	Cys	Arg	Cys	Leu	245	250	255	
Ala	Gln	His	Asp	Phe	Gly	Phe	Ser	Ser	His	Leu	Gly	Asp	Ser	Asp	Thr	260	265	270	
Ser	Glu	Gly	Phe	His	Ile	Cys	Gly	Pro	Asn	Lys	Glu	Leu	Asp	Glu	Glu	275	280	285	

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Thr Cys Gln Cys Val Cys Lys Gly Gly Val Arg Pro Ile Ser Cys Gly
 290 295 300

Pro His Lys Glu Leu Asp Arg Ala Ser Cys Gln Cys Met Cys Lys Asn
 305 310 315 320

Lys Leu Leu Pro Ser Ser Cys Gly Pro Asn Lys Glu Phe Asp Glu Glu
 325 330 335

Lys Cys Gln Cys Val Cys Lys Lys Thr Cys Pro Lys His His Pro Leu
 340 345 350

Asn Pro Ala Lys Cys Ile Cys Glu Cys Thr Glu Ser Pro Asn Lys Cys
 355 360 365

Phe Leu Lys Gly Lys Arg Phe His His Gln Thr Cys Ser Cys Tyr Arg
 370 375 380

Pro Pro Cys Thr Val Arg Thr Lys Arg Cys Asp Ala Gly Phe Leu Leu
 385 390 395 400

Ala Glu Glu Val Cys Arg Cys Val Arg Thr Ser Trp Lys Arg Pro Leu
 405 410 415

Met Asn

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ala Val Val Met Thr Gln Thr Pro Ala Ser
 1 5 10

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCTCTTCTGT GCTTGAGTTG AG

22

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TCTCTTCTGT CCCTGAGTTG AG

22

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 65 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TGTGCTGCAG CAAATTTTAT AGTCTCTTCT GTGGCGGCGG CGGCGGCGGG CGCCTCGCGA

60

GGACC

65

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTGGCAGGGA ACTGCTAATA ATGGAATGAA

30

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(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 84 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGGCTCCGCG TCCGAGAGGT CGAGTCCGGA CTCGTGATGG TGATGGTGAT GGGCGGCGGC 60
GGCGGCGGGC GCCTCGCGAG GACC 84

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 31 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTATTATAAT GTCCTCCACC AAATTTTATA G 31

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 93 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GTTTCGCTGCC TGACACTGTG GTAGTGTTGC TGGCGGCCGC TAGTGATGGT GATGGTGATG 60
AATAATGGAA TGAAC TTGTC TGTAACATC CAG 93

(2) INFORMATION FOR SEQ ID NO:22:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CATGTACGAA CCGCCAGG

18

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AATGACCAGA GAGAGGCGAG

20

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GCCACGGTAG GTCTGCGT

18

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TTTCTTTGAC AGGCTTAT

18

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ATCTTGAAAA GTAAGTATGG G

21

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATGACTTGAC AGGTATTGAT

20

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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AGCAAGACGG TGGGTATTGT

20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCCTTCTTTG TAGTTATTTG AA

22

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CCACAGTGAG TATGAATTAA

20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TTCTTCCAAA GGTGTCAG

18

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GGAGATGGTA GCAGAATG

18

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CTATTTGTCT AGACTCAACA GAT

23

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CAAACATGCA GGTAAGAGAT CC

22

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TGTTCTCCTA GCTGTTACAG A

21

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGCGAGGTCA AGGTAGGTGC AAGG

24

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ATTGTCTTTG ACAGGCTTTT TGAAGG

26

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GAGATCCTGA AAAGTAAGTA G

21

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(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

TGTGACTCGA CAGGTATTGA TAAT

24

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CTCAGCAAGA CGGTAGGTAT

20

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TTGTCCCTTG TAGTTGTTTG AAATT

25

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

ACATTACCAC AGTGAGTATG

20

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GTCTCCCCAA AAGGTGTCAG GCAGCT

26

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

AATGTTGAAG ATGGTAAGTA AAA

23

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

TCTAGACTCA ACCAAT

16

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CAAACATGCA GGTAAGGAGT GT

22

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

TTTTCCCCTA GTTGTTACAG AAGA

24

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2991 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GTTTTAAGTA GAGACGGGGT TTCACCAACG GTTGAAAATA TTTATCATGG TCTCCCTAAG 60

ATGGACGGTG TTAGCTAGGA TGGTCTCGAT CTCCTGACCT CATGATCCAC CCGCCTCGGC 120

CTCCCAAAGT GCTGGGATTA CAGGCGTGAG CCACCGTGTC CGACCAACCT TAAGACAAAC 180

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AACTACTGCA	TGATTGTTTT	TGGAGACCTT	TTTTTTATTC	AAATAAAATTT	TTGCCAGCAT	240
TTTCTGACTC	AAAGTATAGC	AGCAGGAAGA	TAACACTTTT	GTGAGAAAAA	AGTTTGAATA	300
CAGCTTACTG	CTGTATTTAA	ATGAAACAGT	AGTTAATATG	ATATTAATAT	ATTTTGGATA	360
TATTTTGAGT	TTGTTGATTT	TCCAGTCTTC	ACCCGCTGCT	AGGCCTGTGG	GTGTTGGAAA	420
TGCCTGTGTT	TCTCAATTTT	GTTTGCCTAT	TAGAATCCTG	ATGTCCAAGC	CTTACTCCAG	480
TTAGACCAGT	TAAGCCAGAA	AGGCAGAAGG	TGTACTCAAG	CATCTGTTTT	TTCAAAATCT	540
CCTTTTGTGA	TGCCAAGTGC	AATCAAAGTT	TAGAATCATT	GTAATAGCAA	ATGGTTGAAT	600
GGAAACTCCA	CCTTCTATTC	AAATCCTACC	CCAGTCTGCC	CTTAGCTGTT	CTCTTTTCAC	660
AGATCTATCA	ATGTCTGAAG	ATAACTATGG	CAGGCTGATC	AAATATGCAT	AGAGCAGGAA	720
GACAGCAAGA	GAGTGATACA	CTGACCATGT	TCCAAATCAC	AAAACATCTC	AACAGGCTAG	780
ATCATGGACC	GAGTCTGATG	GGATGGAATT	TCATAAAGAT	ACATAAAAAA	GCATCTTGGA	840
TACAGTAAAC	TTAACTCCAC	AAATACAGGG	GAATTTAGAC	GTGACTAAGT	AGCAGTACAT	900
ATGAAAAATT	ATTGAGGAAT	TTTGTGACT	TTAAGGGTAG	TGTGAGTCAA	CACTGTGATT	960
TGGCTGCCAG	AAAAATAAACT	CAATCCAAGG	CTGTATCAAC	AAAGGCATAC	TGTCCATTCT	1020
GCATGCTCAT	TACAGCACTA	AGTACCGAGC	CATGTTCTCA	ACCGCATACT	TCATGAACAT	1080
GGAAAGCTAA	CAGTATGGTT	AAGGGGGGAA	ACTGGAACTG	TCATCTTGGG	GAATAAAAGG	1140
GATATTTAGC	CAGGAGTAAA	GTTAGCTTAG	GGAGACCATG	ATAAATATTT	TCAAAATATT	1200
TGAAGGACTC	AGTTGTGGAA	GTGAGATTAG	ATTTATTGTG	TAAAACTCCA	GGAGTCAAAA	1260
GCAATAGAGA	GATAGAAGGA	AATGCTTTTC	AGCAGTGTTG	CTCATCAATA	AAGGGAGTGA	1320
ACAGCCACAC	AGAATGGAAG	GTTCCCTGTC	CTTTGAGATA	TTTAAGCCTT	CAAGTAAATT	1380
ATGGGTGAGG	AGTTTCAAAT	CTAGAGTTGA	ACCAGATAAG	AAAGTCTCTT	CTTCCGGTAA	1440
GATATTATGG	ACCTATAACA	TCTGTGTACT	TAAAAGTAGA	TTGGGAGTGA	AAGGCAGACT	1500
TTTGATGTTT	TGTACACTGT	TGAAACCCCT	TAGCGTGGTC	CTCTGTAACC	TGCTCACCCCT	1560
GCCCCAAGGA	GGCAGCTAGC	CAATGCCACC	AGCCCCAACGG	AAACCCCAGT	GCTTTTCCAA	1620
TGGGGAAATG	CAGTCACTTT	TCTTTGGATG	CTACACATCC	TTTCTGGAAT	ATGTCTCACA	1680
CACATCTCTC	TTTATCACCC	CCTTTTTCAC	GTAAACCAAC	TTCTTGACAG	AGCTGACAAT	1740
GTGTCTCTTT	ACTCTCCACG	AAGATTCTGG	CCCTTCTCTT	CACCTGTCAG	AAGTTTAGGA	1800
TTCCAAAGGG	ATCATTAGCA	TCCATCCCAA	CAGCCTGCAC	TGCATCCTGA	GAAGTGCGGT	1860
TCTTGGATCA	TCAGGCAACT	TTCAACTACA	CAGACCAAGG	GAGAGAGGGG	ACCCCTCCGA	1920

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GGTCCCATAG	GGTTCTCTGA	CATAGTGATG	ACCTTTTTTC	AAACTTTGAG	CAGGGCGCTG	1980
GGGGCCAGGC	GTGCGGGAGG	GAGGACAAGA	ACTCGGGAGT	GGCCGAGGAT	AAAGCGGGGG	2040
CTCCCTCCAC	CCCACGGTGC	CCAGTTTCTC	CCCGCTGCAC	GTGGTCCAGG	GTGGTCGCAT	2100
CACCTCTAAA	GCCGGTCCCG	CCAACCGCCA	GCCCCGGGAC	TGAACTTGCC	CCTCCGGCCG	2160
CCCGCTCCCC	GCAGGGGACA	GGGGCGGGGA	GGGAGAGATC	CAGAGGGGGG	CTGGGGGAGG	2220
TGGGGCCGCC	GGGGAGGAGG	CGAGGGAAAC	GGGGAGCTCC	AGGGAGACGG	CTTCCGAGGG	2280
AGAGTGAGAG	GGGAGGGCAG	CCCGGGCTCG	GCACGCTCCC	TCCCTCGGCC	GCTTTCTCTC	2340
ACATAAGCGC	AGGCAGAGGG	CGCGTCAGTC	ATGCCCTGCC	CCTGCGCCCG	CCGCCGCCGC	2400
CGCCGCCGCT	CAGCCCGGCG	CGCTCTGGAG	GATCCTGCGC	CGCGGCGCTC	CCGGGCCCCG	2460
CCGCCGCCAG	CCGCCCCGGC	GGCCCTCCTC	CCGCCCCCGG	CACCGCCGCC	AGCGCCCCCG	2520
CCGCAGCGCC	CGCGGCCCGG	CTCCTCTCAC	TTCGGGAAG	GGGAGGGAGG	AGGGGGACGA	2580
GGGCTCTGGC	GGGTTTGGAG	GGGCTGAACA	TCGCGGGGTG	TTCTGGTGTC	CCCCGCCCCG	2640
CCTCTCAAAA	AAGCTACACC	GACGCGGACC	GCGGCGGCGT	CCTCCCTCGC	CCTCGCTTCA	2700
CCTCGCGGGC	TCCGAATGCG	GGGAGCTCGG	ATGTCCGGTT	TCCTGTGAGG	CTTTTACCTG	2760
ACACCCGCCG	CCTTTCCCCG	GCACTGGCTG	GGAGGGCGCC	CTGCAAAGTT	GGGAACGCGG	2820
AGCCCCGGAC	CCGCTCCCGC	CGCCTCCGGC	TCGCCCAGGG	GGGGTCGCCG	GGAGGAGCCC	2880
GGGGGAGAGG	GACCAGGAGG	GGCCCGCGGC	CTCGCAGGGG	CGCCCGCGCC	CCCACCCCTG	2940
CCCCCGCCAG	CGGACCGGTC	CCCCACCCCC	GGTCCTTCCA	CCATGCACTT	G	2991

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CACGGCTTAT GCAAGCAAAG

20

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

AACACAGTTT TCCATAATAG

20

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Leu Ser Lys Thr Val Ser Gly Ser Glu Gln Asp Leu Pro His Glu Leu
1 5 10 15
His Val Glu

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GACGGACACA GATGGAGGTT TAAAG

25

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 196 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

```

Met Arg Thr Leu Ala Cys Leu Leu Leu Leu Gly Cys Gly Tyr Leu Ala
1           5           10           15

His Val Leu Ala Glu Glu Ala Glu Ile Pro Arg Glu Val Ile Glu Arg
20           25           30

Leu Ala Arg Ser Gln Ile His Ser Ile Arg Asp Leu Gln Arg Leu Leu
35           40           45

Glu Ile Asp Ser Val Gly Ser Glu Asp Ser Leu Asp Thr Ser Leu Arg
50           55           60

Ala His Gly Val His Ala Thr Lys His Val Pro Glu Lys Arg Pro Leu
65           70           75           80

Pro Ile Arg Arg Lys Arg Ser Ile Glu Glu Ala Val Pro Ala Val Cys
85           90           95

Lys Thr Arg Thr Val Ile Tyr Glu Ile Pro Arg Ser Gln Val Asp Pro
100          105          110

Thr Ser Ala Asn Phe Leu Ile Trp Pro Pro Cys Val Glu Val Lys Arg
115          120          125

Cys Thr Gly Cys Cys Asn Thr Ser Ser Val Lys Cys Gln Pro Ser Arg
130          135          140

Val His His Arg Ser Val Lys Val Ala Lys Val Glu Tyr Val Arg Lys
145          150          155          160

Lys Pro Lys Leu Lys Glu Val Gln Val Arg Leu Glu Glu His Leu Glu
165          170          175

Cys Ala Cys Ala Thr Thr Ser Leu Asn Pro Asp Tyr Arg Glu Glu Asp
180          185          190

Thr Asp Val Arg
195

```

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 241 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Met	Asn	Arg	Cys	Trp	Ala	Leu	Phe	Leu	Ser	Leu	Cys	Cys	Tyr	Leu	Arg	1	5	10	15
Leu	Val	Ser	Ala	Glu	Gly	Asp	Pro	Ile	Pro	Glu	Glu	Leu	Tyr	Glu	Met	20	25	30	
Leu	Ser	Asp	His	Ser	Ile	Arg	Ser	Phe	Asp	Asp	Leu	Gln	Arg	Leu	Leu	35	40	45	
His	Gly	Asp	Pro	Gly	Glu	Glu	Asp	Gly	Ala	Glu	Leu	Asp	Leu	Asn	Met	50	55	60	
Thr	Arg	Ser	His	Ser	Gly	Gly	Glu	Leu	Glu	Ser	Leu	Ala	Arg	Gly	Arg	65	70	75	80
Arg	Ser	Leu	Gly	Ser	Leu	Thr	Ile	Ala	Glu	Pro	Ala	Met	Ile	Ala	Glu	85	90	95	
Cys	Lys	Thr	Arg	Thr	Glu	Val	Phe	Glu	Ile	Ser	Arg	Arg	Leu	Ile	Asp	100	105	110	
Arg	Thr	Asn	Ala	Asn	Phe	Leu	Val	Trp	Pro	Pro	Cys	Val	Glu	Val	Gln	115	120	125	
Arg	Cys	Ser	Gly	Cys	Cys	Asn	Asn	Arg	Asn	Val	Gln	Cys	Arg	Pro	Thr	130	135	140	
Gln	Val	Gln	Leu	Arg	Pro	Val	Gln	Val	Arg	Lys	Ile	Glu	Ile	Val	Arg	145	150	155	160
Lys	Lys	Pro	Ile	Phe	Lys	Lys	Ala	Thr	Val	Thr	Leu	Glu	Asp	His	Leu	165	170	175	
Ala	Cys	Lys	Cys	Glu	Thr	Val	Ala	Ala	Ala	Arg	Pro	Val	Thr	Arg	Ser	180	185	190	
Pro	Gly	Gly	Ser	Gln	Glu	Gln	Arg	Ala	Lys	Thr	Pro	Gln	Thr	Arg	Val	195	200	205	
Thr	Ile	Arg	Thr	Val	Arg	Val	Arg	Arg	Pro	Pro	Lys	Gly	Lys	His	Arg	210	215	220	
Lys	Phe	Lys	His	Thr	His	Asp	Lys	Thr	Ala	Leu	Lys	Glu	Thr	Leu	Gly	225	230	235	240
Ala																			

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- 141 -

- (A) LENGTH: 149 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
1           5           10           15

Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
          20           25           30

Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
          35           40           45

Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
50           55           60

Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
65           70           75           80

Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro
          85           90           95

Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
          100          105          110

Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
115          120          125

Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Cys Gly Asp
130          135          140

Ala Val Pro Arg Arg
145

```

(2) INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 191 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: not relevant
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

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Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
 1 5 10 15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
 20 25 30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
 35 40 45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
 50 55 60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
 65 70 75 80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
 85 90 95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
 100 105 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
 115 120 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
 130 135 140

Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
 145 150 155 160

Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
 165 170 175

Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
 180 185 190

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 188 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
 1 5 10 15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
 20 25 30

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```

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
 35          40          45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
 50          55          60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
 65          70          75          80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
      85          90          95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
      100          105          110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
 115          120          125

Lys Asp Ser Ala Val Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro Arg
 130          135          140

Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg
 145          150          155          160

Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu Leu
      165          170          175

Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg
      180          185

```

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 419 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: other
- (B) LOCATION: 156
- (D) OTHER INFORMATION: /note= "codon 156 can be anything other than cysteine, or can be nothing"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

```

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
 1          5          10          15

```

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Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe
 20 25 30
 Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
 35 40 45
 Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
 50 55 60
 Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
 65 70 75 80
 Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
 85 90 95
 Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
 100 105 110
 His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
 115 120 125
 Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
 130 135 140
 Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Xaa Val Ser Val Tyr
 145 150 155 160
 Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
 165 170 175
 Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
 180 185 190
 Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
 195 200 205
 Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
 210 215 220
 Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
 225 230 235 240
 Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
 245 250 255
 Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
 260 265 270
 Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
 275 280 285
 Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
 290 295 300
 Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
 305 310 315 320

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Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
 325 330 335
 Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
 340 345 350
 Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
 355 360 365
 Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
 370 375 380
 Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
 385 390 395 400
 Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
 405 410 415
 Gln Met Ser

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 160 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
 1 5 10 15
 Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Thr
 20 25 30
 Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu
 35 40 45
 Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu
 50 55 60
 Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe
 65 70 75 80
 Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn
 85 90 95
 Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys
 100 105 110

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Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val
115 120 125

Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu
130 135 140

Asp Val Tyr Arg Gln Val His Ser Ile Ile His His His His His
145 150 155 160

CLAIMS

1. A purified and isolated polypeptide selected from the group consisting of:
 - (a) a purified and isolated polypeptide capable of binding to at least one of KDR receptor tyrosine kinase (VEGFR-2) and Flt4 receptor tyrosine kinase (VEGFR-3), said polypeptide comprising a portion of the amino acid sequence in SEQ ID NO: 8 effective to permit such binding;
 - (b) a purified and isolated VEGF-C of vertebrate origin, wherein said VEGF-C has a molecular weight of about 21-23 kD or about 30-32 kD, as assessed by SDS-PAGE under reducing conditions, and wherein said VEGF-C is capable of binding to Flt4 receptor tyrosine kinase (VEGFR-3);
 - (c) a purified polypeptide analog of human VEGF-C that is capable of binding to at least one of Flt-1 receptor tyrosine kinase (VEGFR-1), KDR receptor tyrosine kinase (VEGFR-2), and Flt4 receptor tyrosine kinase (VEGFR-3); and
 - (d) a polypeptide analog of human VEGF that is capable of binding to at least one of VEGFR-1, VEGFR-2, and VEGFR-3, wherein a cysteine residue is introduced in the VEGF amino acid sequence at a position selected from residues 53 to 63 of the human VEGF165 precursor having the amino acid sequence set forth in SEQ ID NO: 56.
2. A purified and isolated polypeptide according to claim 1 that is capable of binding to at least one of KDR receptor tyrosine kinase (VEGFR-2) and Flt4 receptor tyrosine kinase (VEGFR-3), said polypeptide comprising a portion of the amino acid sequence in SEQ ID NO: 8 effective to permit such binding.
3. A polypeptide according to claim 1 or 2, wherein said polypeptide is capable of stimulating tyrosine phosphorylation of a receptor selected from the group consisting of VEGFR-2 and VEGFR-3 in a host cell expressing said receptor.
4. A purified and isolated polypeptide multimer, wherein at least one monomer thereof is a polypeptide according to any one of claims 1-3, and wherein said multimer is capable of binding to at least one of VEGFR-2 and VEGFR-3.

5. A multimer according to claim 4 having a VEGF-C biological activity.
6. A multimer according to claim 4 or 5 wherein at least one monomer thereof is selected from the group consisting of a vascular endothelial growth factor (VEGF) polypeptide, a vascular endothelial growth factor B (VEGF-B) polypeptide, a platelet derived growth factor A (PDGF-A) polypeptide, a platelet derived growth factor B (PDGF-B) polypeptide, a *c-fos* induced growth factor (FIGF) polypeptide, and a placenta growth factor (PlGF) polypeptide.
7. A dimer according to claim 4, 5, or 6.
8. A dimer according to claim 7 wherein each monomer thereof is capable of binding to at least one of VEGFR-2 and VEGFR-3 and has an amino acid sequence comprising a portion of SEQ ID NO: 8 effective to permit such binding.
9. A dimer according to claim 7 or 8 wherein the two monomers are free of covalent attachments to each other.
10. A purified and isolated polypeptide according to claim 1 that is a VEGF-C of vertebrate origin, wherein said VEGF-C has a molecular weight of about 21-23 kD, as assessed by SDS-PAGE under reducing conditions, and wherein said VEGF-C is capable of binding to Flt4 receptor tyrosine kinase (VEGFR-3).
11. A purified and isolated polypeptide according to claim 1 that is a VEGF-C of vertebrate origin, wherein said VEGF-C has a molecular weight of about 30-32 kD, as assessed by SDS-PAGE under reducing conditions, and wherein said VEGF-C is capable of binding to Flt4 receptor tyrosine kinase (VEGFR-3).

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12. A polypeptide analog of the VEGF-C according to claim 10 or 11, wherein a conserved cysteine residue in said VEGF-C has been deleted or replaced, and wherein said analog is capable of binding to VEGFR-3 and has reduced VEGFR-2 binding affinity relative to said VEGF-C.

13. A polypeptide analog according to claim 10 or 11 wherein said conserved cysteine residue corresponds to the cysteine at position 156 of SEQ ID NO: 8.

14. A purified polypeptide according to claim 1 that is an analog of human VEGF-C that is capable of binding to at least one of Flt-1 receptor tyrosine kinase (VEGFR-1), KDR receptor tyrosine kinase (VEGFR-2), and Flt4 receptor tyrosine kinase (VEGFR-3).

15. A polypeptide according to claim 14 that binds VEGFR-3 and has reduced VEGFR-2 binding affinity relative to human VEGF-C having an amino acid sequence consisting essentially of amino acids 103-227 of SEQ ID NO: 8.

16. A polypeptide according to claim 14 or 15 that is a VEGF-C ΔC_{156} polypeptide.

17. A VEGF-C ΔC_{156} polypeptide according to claim 16 comprising amino acids 131 to 211 of SEQ ID NO: 8, wherein the cysteine residue at position 156 of SEQ ID NO: 8 has been deleted or replaced.

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18. A VEGF-C ΔC_{156} polypeptide according to claim 16 or 17 comprising a continuous portion of SEQ ID NO: 8, said portion having as its amino terminal residue an amino acid between residues 102 and 114 of SEQ ID NO: 8, and having as its carboxy terminal residue an amino acid between residues 212 and 228 of SEQ ID NO: 8, wherein the cysteine residue at position 156 of SEQ ID NO: 8 has been deleted or replaced.

19. A VEGF-C ΔC_{156} polypeptide according to any one of claims 16-18 wherein the cysteine residue at position 156 of SEQ ID NO: 8 has been replaced by a serine residue.

20. A polypeptide according to claim 14 or 15 that is a VEGF-C $\Delta R_{226}\Delta R_{227}$ polypeptide.

21. A VEGF-C $\Delta R_{226}\Delta R_{227}$ polypeptide according to claim 20 having an amino acid sequence comprising amino acids 112-419 of SEQ ID NO: 8, wherein the arginine residues at positions 226 and 227 of SEQ ID NO: 8 have been deleted or replaced.

22. A polypeptide according to claim 14 that is a human VEGF-C^{basic} polypeptide.

23. A polypeptide according to claim 22 having an amino acid sequence comprising residues 131 to 211 of SEQ ID NO: 8, wherein the glutamic acid residue at position 187, the threonine residue at position 189, and the proline residue at position 191 of SEQ ID NO: 8 have been replaced by an arginine residue, a lysine residue, and a histidine residue, respectively.

24. A composition comprising a polypeptide according to any one of claims 1-4 and 10-23, and further comprising a purified myelopoietic growth factor in admixture therewith.

25. A kit useful for modulating myelopoiesis comprising: a first composition comprising a polypeptide according to any one of claims 1-4 and 10-23, packaged with at least one additional composition comprising a myelopoietic growth factor.

26. A composition according to claim 24 or a kit according to claim 25 wherein the myelopoietic growth factor is selected from the group consisting of granulocyte colony stimulating factor (G-CSF), macrophage-CSF (M-CSF), granulocyte-macrophage-CSF (GM-CSF), interleukin-3 (IL-3), stem cell factor (SCF), and combinations thereof.

27. A composition comprising a polypeptide according to any one of claims 1-4 and 10-23, and further comprising a purified polypeptide selected from the group consisting of vascular endothelial growth factor (VEGF) polypeptides, vascular endothelial growth factor B (VEGF-B) polypeptides, platelet derived growth factor A (PDGF-A) polypeptides, platelet derived growth factor B (PDGF-B) polypeptides, *c-fos* induced growth factor (FIGF) polypeptides, and placenta growth factor (PIGF) polypeptides.

28. A polypeptide according to claim 1 that is an analog of human VEGF, wherein a cysteine residue is introduced in the VEGF amino acid sequence at a position selected from residues 53 to 63 of the human VEGF165 precursor having the amino acid sequence set forth in SEQ ID NO: 56, and wherein the polypeptide is capable of binding to at least one of VEGFR-1, VEGFR-2, and VEGFR-3.

29. An analog according to claim 28 wherein said cysteine is introduced at position 58 of the VEGF165 precursor having the amino acid sequence set forth in SEQ ID NO: 56.

30. A purified and isolated nucleic acid comprising a nucleotide sequence encoding a polypeptide according to any one of claims 1-4, 10-23, and 28-29.

31. A vector comprising a nucleic acid according to claim 30.
32. A host cell transformed or transfected with a nucleic acid according to claim 30 or a vector according to claim 31.
33. A method of making a polypeptide capable of specifically binding to at least one of VEGFR-1, VEGFR-2, and VEGFR-3, said method comprising the steps of:
- (a) expressing a nucleic acid according to claim 30 or a vector according to claim 31 in a host cell; and
 - (b) purifying a polypeptide capable of specifically binding to at least one of VEGFR-1, VEGFR-2, and VEGFR-3 from said host cell or from a growth medium of said host cell.
34. An antibody which is specifically reactive with a polypeptide according to any one of claims 1-4, 10-23, and 28-29.
35. A pharmaceutical composition comprising an antibody according to claim 34 in a pharmaceutically-acceptable diluent, adjuvant, excipient, or carrier.
36. A pharmaceutical composition comprising a polypeptide according to any one of claims 1-4, 10-23, and 28-29 in a pharmaceutically-acceptable diluent, adjuvant, excipient, or carrier.
37. A method of modulating the proliferation of mammalian endothelial cells comprising the step of contacting mammalian endothelial cells with a polypeptide in an amount effective to modulate the proliferation of mammalian endothelial cells, said polypeptide selected from the group consisting of:
- (a) a polypeptide according to any one of claims 1-4, 10-23, and 28-29; and
 - (b) a polypeptide comprising an antigen binding portion of an anti-VEGF-C antibody.

38. A method of increasing the proliferation of mammalian endothelial cells according to claim 37, comprising contacting mammalian endothelial cells with a polypeptide in an amount effective to increase the proliferation of mammalian endothelial cells.

39. A method according to claim 37 or 38 wherein said endothelial cells are lymphatic endothelial cells.

40. An *in vivo* method according to claim 39 wherein the contacting step comprises administering to a mammalian subject in need of modulation of the proliferation of lymphatic endothelial cells a composition comprising said polypeptide, in an amount effective to modulate the proliferation of lymphatic endothelial cells *in vivo*.

41. A method according to claim 40 wherein said polypeptide has reduced effect on the permeability of mammalian blood vessels compared to a wildtype VEGF-C polypeptide with an amino acid sequence set forth in SEQ ID NO: 8 from residue 103 to residue 227.

42. A method according to any one of claims 37- 41 wherein said polypeptide is a VEGF-C ΔC_{156} polypeptide.

43. A method for modulating myelopoiesis in a mammalian subject comprising administering to a mammalian subject in need of modulation of myelopoiesis an amount of a polypeptide effective to modulate myelopoiesis, said polypeptide selected from the group consisting of:

(a) a polypeptide according to any one of claims 1-4, 10-23, and 28-29;
and

(b) a polypeptide comprising an antigen binding portion of an anti-VEGF-C antibody.

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44. A method according to claim 43 wherein said mammalian subject suffers from granulocytopenia, and said method comprises administering to said subject an amount of a polypeptide effective to stimulate myelopoiesis.

45. A method according to claim 43 or 44 comprising administering to said subject an amount of a polypeptide effective to increase the neutrophil count in blood of said subject.

46. A method according to any one of claims 43-45 wherein said mammalian subject is human.

47. A method according to any one of claims 43-46 further comprising administering to said subject a myelopoietic growth factor selected from the group consisting of granulocyte colony stimulating factor (G-CSF), macrophage-CSF (M-CSF), granulocyte-macrophage-CSF (GM-CSF), interleukin-3 (IL-3), stem cell factor (SCF), and combinations thereof.

48. A method of modulating the proliferation of neutrophilic granulocytes in vitro comprising the step of contacting mammalian stem cells with a polypeptide in an amount effective to modulate the proliferation of neutrophilic granulocytes, said polypeptide selected from the group consisting of:

(a) a polypeptide according to any one of claims 1-4, 10-23, and 28-29;
and

(b) a polypeptide comprising an antigen binding portion of an anti-VEGF-C antibody.

49. A method of modulating the proliferation and/or differentiation of mammalian CD34+ progenitor cells comprising contacting mammalian CD34+ progenitor cells with a polypeptide according to any one of claims 1-4, 10-23, and 28-29, in an amount effective to modulate the proliferation and/or differentiation of the cells.

50. A method according to claim 49 further comprising contacting the mammalian CD34+ progenitor cells with a myelopoietic growth factor selected from the group consisting of granulocyte colony stimulating factor (G-CSF), macrophage-CSF (M-CSF), granulocyte-macrophage-CSF (GM-CSF), interleukin-3 (IL-3), stem cell factor (SCF), and combinations thereof, in an amounts effective to modulate the proliferation of CD34+ progenitor cells when used in combination with said polypeptide.

51. A method according to claim 49 or 50 wherein the contacting is performed *in vitro* by culturing mammalian CD34+ progenitor cells in the presence of the polypeptide and optionally the myelopoietic growth factor.

52. A method of increasing the number of neutrophils in the blood of a mammalian subject comprising the step of expressing in a cell in a subject in need of an increased number of blood neutrophils a DNA encoding a VEGF-C protein, said DNA operatively linked to a non-VEGF-C promoter or other non-VEGF-C control sequence that promotes expression of said DNA in said cell.

53. A method of increasing the number of endothelial cells in a mammalian subject comprising the step of expressing in a cell in a subject in need of an increased number of endothelial cells a DNA encoding a VEGF-C protein, said DNA operatively linked to a non-VEGF-C promoter or other non-VEGF-C control sequence that promotes expression of said DNA in said cell.

54. A cell comprising a nucleic acid having a sequence encoding human VEGF-C and further comprising a non-VEGF-C promoter sequence or other non-VEGF-C control sequence that increases RNA transcription in said cell of said sequence encoding human VEGF-C.

55. A purified nucleic acid comprising a VEGF-C promoter sequence.

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56. A nucleic acid according to claim 55 comprising a portion of SEQ ID NO: 48, wherein said portion is capable of promoting expression of a protein encoding gene operatively linked thereto under conditions wherein VEGF-C is expressed in native host cells.

57. A chimeric nucleic acid comprising a nucleic acid according to claim 55 or 56 operatively connected to a sequence encoding a protein other than a human VEGF-C.

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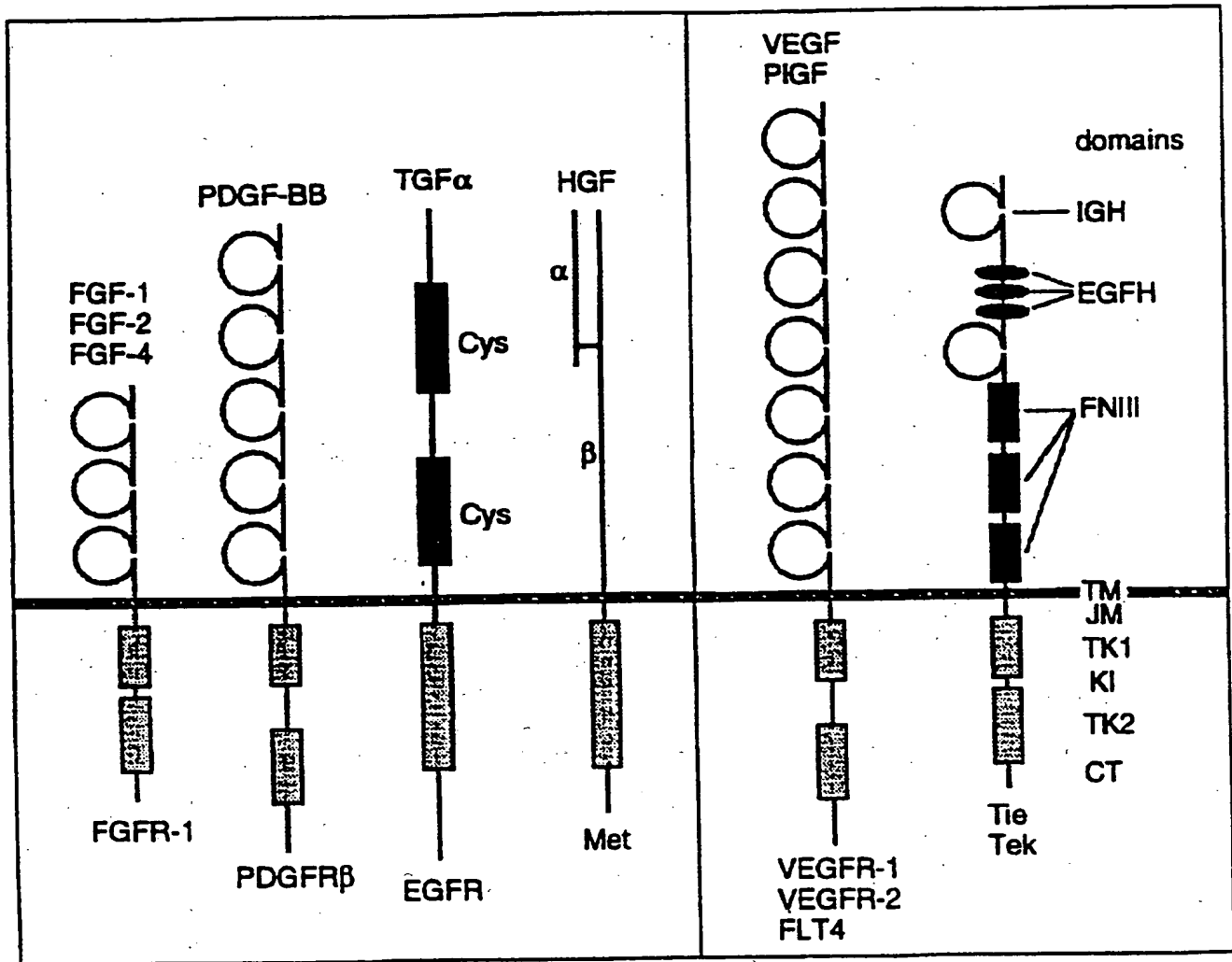


FIGURE 1

1	50
PDGF-AMRTLACLLL
PDGF-B MNRCWA.LFL
PlGF-1
VEGF165
VEGF-B167
VEGF-C
MHLGFFSVA	CSLLAAALLP GPREAPAAA AFESGLDLS AEPDAGEATA
51	100
PDGF-A	LGCGYLAHVL AEEAEIPREV IERLARSQIH SIRDQLRLL IDS VGSEDSL
PDGF-B	SLCCYLRLVS AEGDPIPEEL YEMLSHDSIR SFDDLQRLH GDP.GEEDGA
PlGF-1MPVM RLFPCL..FLQ LLAGLAL... PAVPPQQW..
VEGF165M NFLLS..WVH WSLALLLYLH HAKWSQAA..
VEGF-B167M SPLLR..RL LALLQLAPA QAPVSQP...
VEGF-C	YASKDLEEQL RSVSSVDELM TVLYPEYWK YKCLRKG GW QHNREQANLN
101	150
PDGF-A	DTSLRAHG VH ATKHVPEKRP LPIRRKRSI.EEAVP AVCKTRTRTVIY
PDGF-B	ELDLNMTRSH SGGELES... .LARGRRSLG SLTIAEPAMI AECKTRTRTEVF
PlGF-1ALSAG NGSEVEVVP FQE.VWGR.. SYCRALERLV
VEGF165PMAEG GGQNHHEVVK FMD.VYQR.. SYCHPIETLV
VEGF-B167D APGHQRKVVS WID.VYTR.. ATCQPREVVV
VEGF-C	SRTEETIKFA AAHYNTEILK SIDNEWRK..TQCMPREVCI
151	200
PDGF-A	EIPRSQVDPT SANFLIWPPC VEVKRC TGCC NTSSVKCQPS RVHHRSVKVA
PDGF-B	EISRRLLDRT NANFLVWPPC VEVQRCSGCC NNRNVQCRPT QVQLRPVQVR
PlGF-1	DVSEYPS EV ..EHMFSPC VSLLRCTGCC GDENLHCVPV ETANVTMQLL
VEGF165	DIFQEY PDEI ..EYIFKPS VPLMR CGGCC NDEGLECVPT EESNITMQIM
VEGF-B167	PLTVELMGTV ..AKQLVPSC VTVQR CGGCC PDDGLECVPT GQHQVRMQIL
VEGF-C	DVGKEFGVAT ..NTFFKPPC VSVYRCGGCC NSEGLQCMNT STSYLSKTLF

FIGURE 2 A

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201	PDGF-A	KVEYVRKKPK	LKEVQVRLEE	HLEACAT..	TSLNPDYREE	250
	PDGF-B	KIEIVRKKPI	FKKATVTLED	HLACKCETVA	AARPVTRSPG	GSQEQRAKTP	
	PlGF-1	KIRSG..DRP	.SYVELTFSQ	HVRCECRPLR	EK.....	
	VEGF165	RIKPH..QGQ	.HIGEMSFLQ	HNKCECRPKK	DR.....	
	VEGF-B167	MIRYP..SSQ	.LGEMSLEE	HSQCECRPKK	KD.....	
	VEGF-C	EITVPLSQGP	.KPVTTISFAN	HTSCRCMSKL	DVYRQVHSII	RRSLPATLPQ	
							300
	PDGF-A	DTDVR.....	
	PDGF-B	QTRVTIRTVR	VRRPPKGKHR	KFKHTHDKTA	LKETLGA...	
	PlGF-1	MKPERCGDA	VPRR.....	
	VEGF165ARQENPCGP	CSERRKHLFV	
	VEGF-B167AVKPDSPRPL	CPRCTQHHQR	
	VEGF-C	CQAANKTCPT	NYMWNNHICR	CLAQEDFMFS	SDAGDDSTDG	FHDICGPNKE	
							350
	PDGF-A	
	PDGF-B	
	PlGF-1	
	VEGF165	QDPQTCCKSC	KNTDS.RCKA	RQLELNERTC	RCDKPRR...	
	VEGF-B167	PDPRTCRCRC	RRRSFLRCQG	RGLELNPDTC	RCRKLRR...	
	VEGF-C	LDEETCQCVC	RAGLRPASC	PHKELDRNSC	QCVCKNKLFP	SQCGANREFD	

FIGURE 2 B

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351 400
PDGF-A
PDGF-B
PlGF-1
VEGF165
VEGF-B167
VEGF-C
ENTCQCVCCKR TCPRNQPLNP GKACECTES POKCLLKGGK FHHQTCSCYR

401 434
PDGF-A
PDGF-B
PlGF-1
VEGF165
VEGF-B167
VEGF-C
RPCTNRQKAC EPGFSYSEEV CRCVPSYWK R PQMS

FIGURE 2 C

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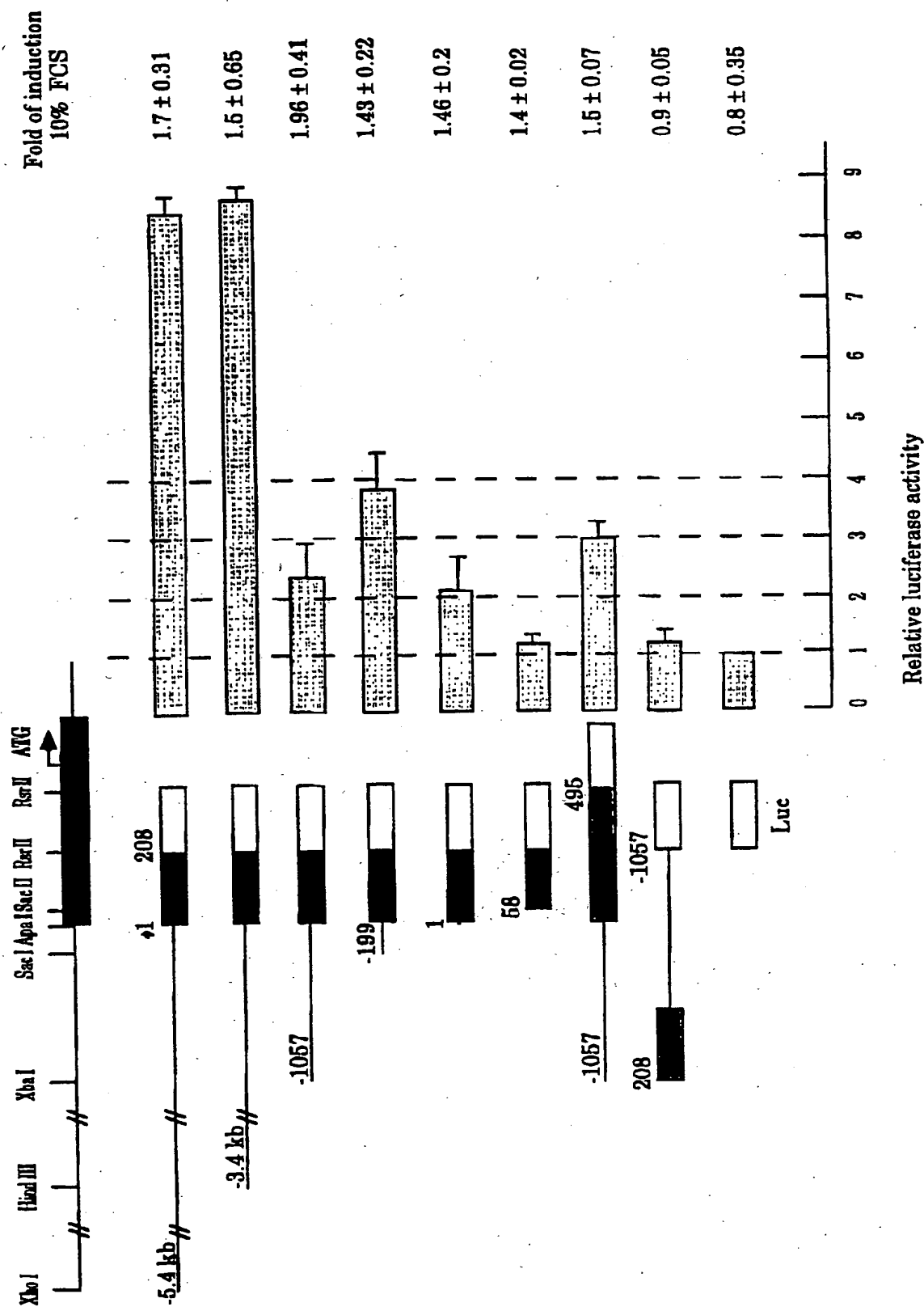


FIGURE 3

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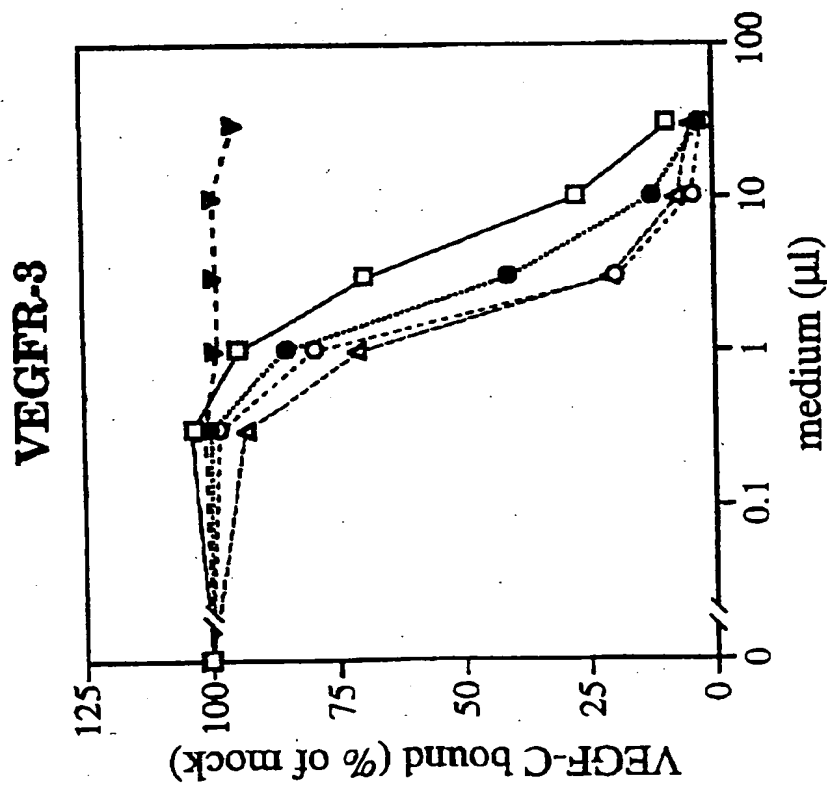
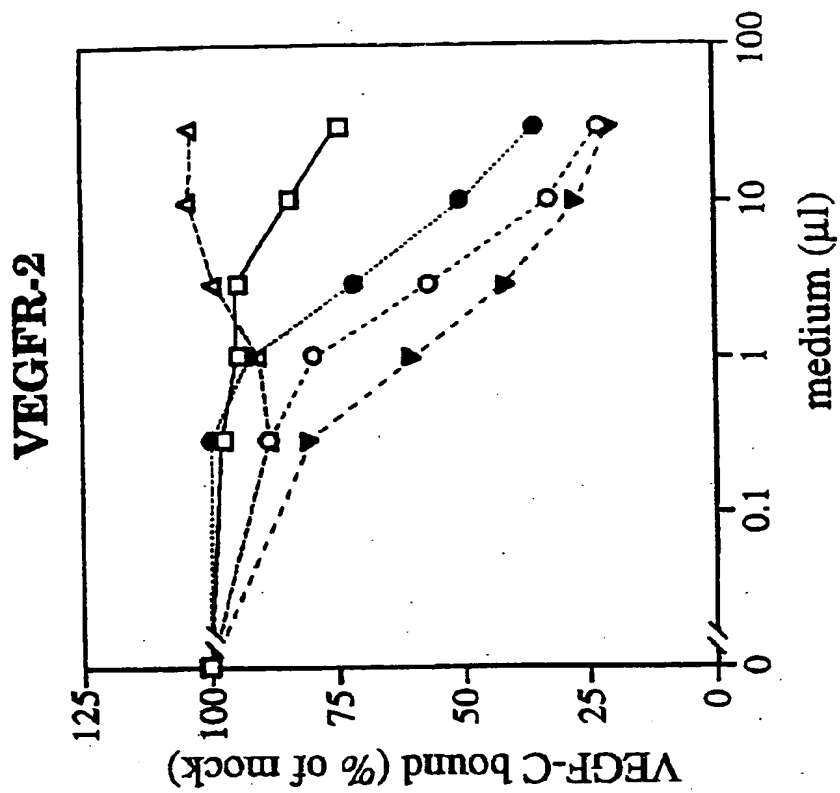


FIGURE 4

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VEGF-C alignment

	1				50
Hum	HMLLGFFSVA	CSLLAAALLP	GPREAPAAAA	AFESGLDLSD	AEPDAGEATA
Mou	MHLLCFLSLA	CSLLAAALIP	SPREAPATVA	AFESGLGFSE	AEPDGGEVKA
Qua	MHLLLEMLSLG	CCLAAGAVLL	GPROPPVA.A	AYESGHGYE	EPPGAGEPKA
	51				100
Hum	YASKDLEEQL	RSVSSVDELM	TVLYPEYWKM	YKCQLRKGGW	QHNREQANLN
Mou	FEGKDLEEQL	RSVSSVDELM	SVLYPDYWKM	YKCQLRKGGW	Q....QPTLN
Qua	HASKDLEEQL	RSVSSVDELM	TVLYPEYWKM	FKCQLRKGGW	QHNREHSSSD
	101				150
Hum	SRTEETIKFA	AAHYNTEILK	SIDNEWRTQ	CMPREVCIDV	GKEFGVATNT
Mou	TRTGDSVKFA	AAHYNTEILK	SIDNEWRTQ	CMPREVCIDV	GKEFGAATNT
Qua	TRSDDSLKFA	AAHYNAEILK	SIDTEWRTQ	GMPREVCVDL	GKEFGATTNT
	151				200
Hum	FFKPPCVSVY	RCGGCCNSEG	LQCMNTSTSY	LSKTLFEITV	PLSQGPKPVT
Mou	FFKPPCVSVY	RCGGCCNSEG	LQCMNTSTGY	LSKTLFEITV	PLSQGPKPVT
Qua	FFKPPCVSIY	RCGGCCNSEG	LQCMNISTNY	ISKTLFEITV	PLSHGPKPVT
	201				250
Hum	ISFANHTSCR	CMSKLDVYRQ	VHSIIRSLP	ATLPQCOAAN	KTCPTNYMWN
Mou	ISFANHTSCR	CMSKLDVYRQ	VHSIIRSLP	ATLPQCOAAN	KTCPTNYVWN
Qua	VSFANHTSCR	CMSKLDVYRQ	VHSIIRSLP	ATQTQCHVAN	KTCPKNHVWN
	251				300
Hum	NHICRCLAQE	DFMFSSDAGD	DSTDGFHDIC	GPNKELDEET	CQCVCRAGLR
Mou	NYMCRCCLAQQ	DFIFYSNVED	DSTNGFHDVC	GPNKELDEDT	CQCVCCKGGLR
Qua	NQICRCLAQH	DFGFSSHLGD	SDTSEGFHIC	GPNKELDEET	CQCVCCKGGVR
	301				350
Hum	PASCGPHKEL	DRNSCQCVCK	NKLFPSCGA	NREFDENTCQ	CVCKRTCPRN
Mou	PSSCGPHKEL	DRDESCQCVCK	NKLFPNSCGA	NREFDENTCQ	CVCKRTCPRN
Qua	PISCGPHKEL	DRASCQCMCK	NKLLPSSCGP	NKEFDEEKCO	CVCKKTCPKH
	351				400
Hum	QPLNPGKCAC	ECTESPQKCL	LKGKKFHHQT	CSCYRRPCTN	RQKACEPGFS
Mou	QPLNPGKCAC	ECTENTQKCF	LKGKKFHHQT	CSCYRRPCAN	RLKHCDPGLS
Qua	HPLNPAKCIC	ECTESPNKCF	LKGKRFHHQT	CSCYRPPCTV	RTKRCDAGFL
	401		420		
Hum	YSEEVCRCPV	SYWKRPQMS*			
Mou	FSEEVCRCPV	SYWKRPHLN.			
Qua	LAEEVCRCVR	TSWKRPPLMN*			

FIGURE 5

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FIGURE 6A

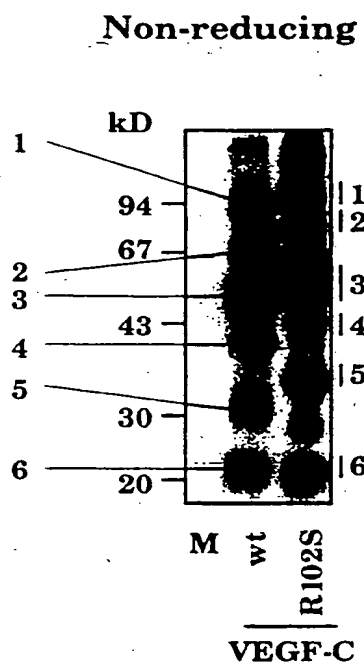


FIGURE 6B

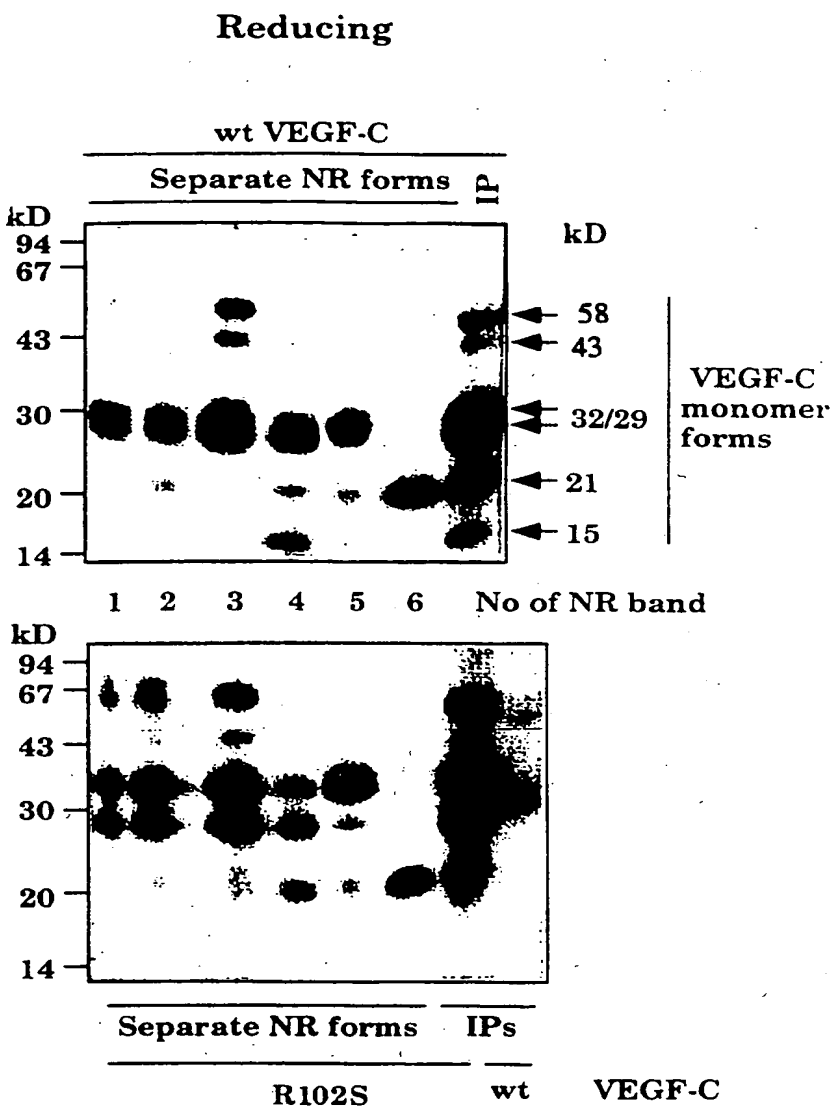


FIGURE 6C

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Media

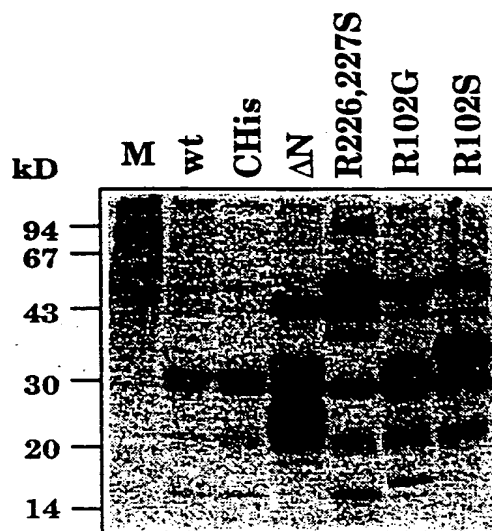


FIGURE 7A

Lysates

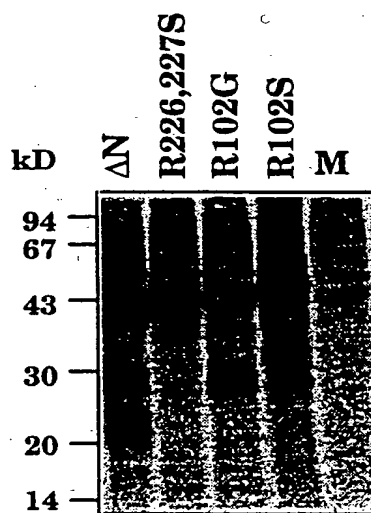


FIGURE 7B

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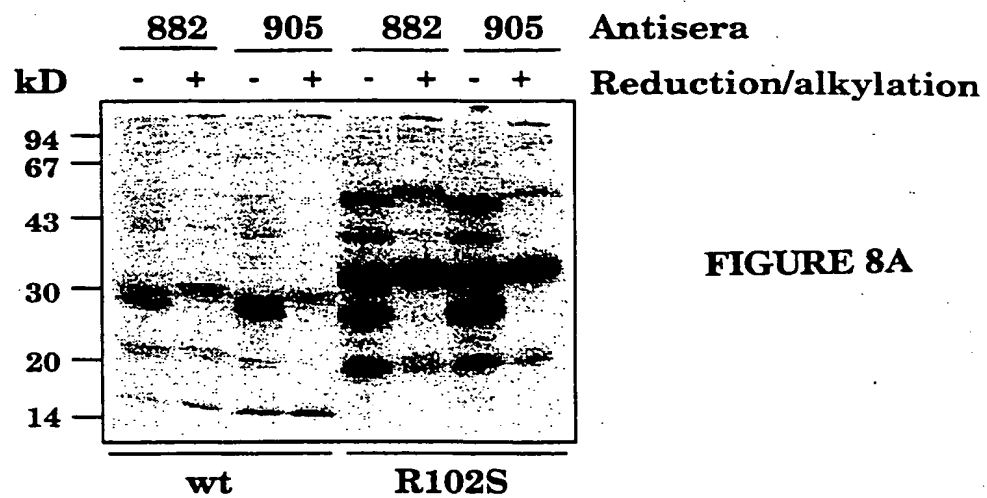


FIGURE 8A

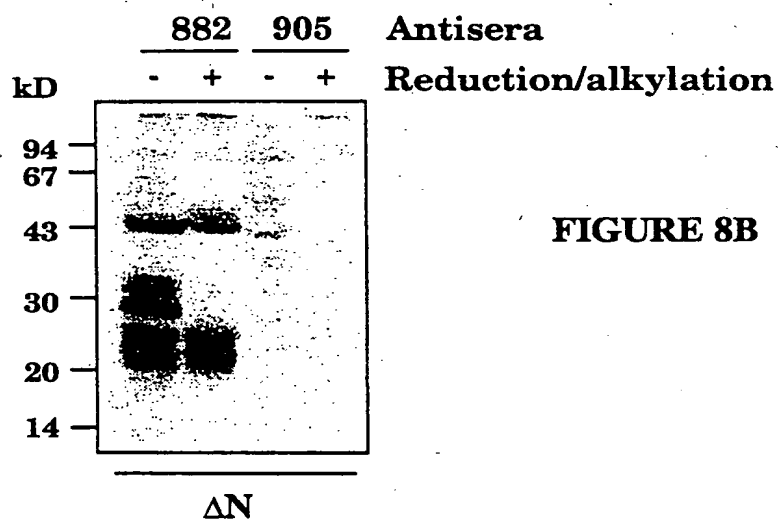


FIGURE 8B

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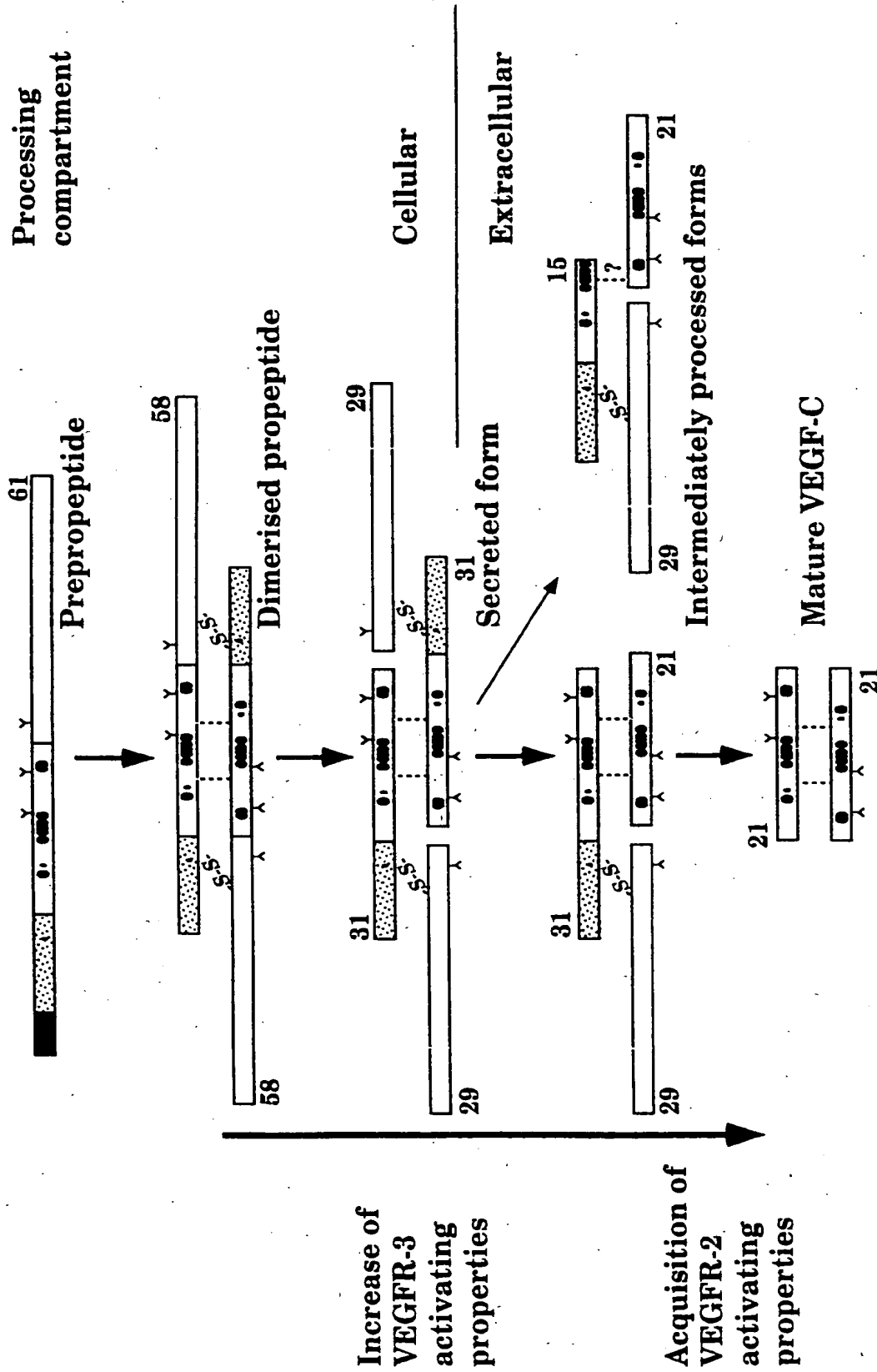


FIGURE 9

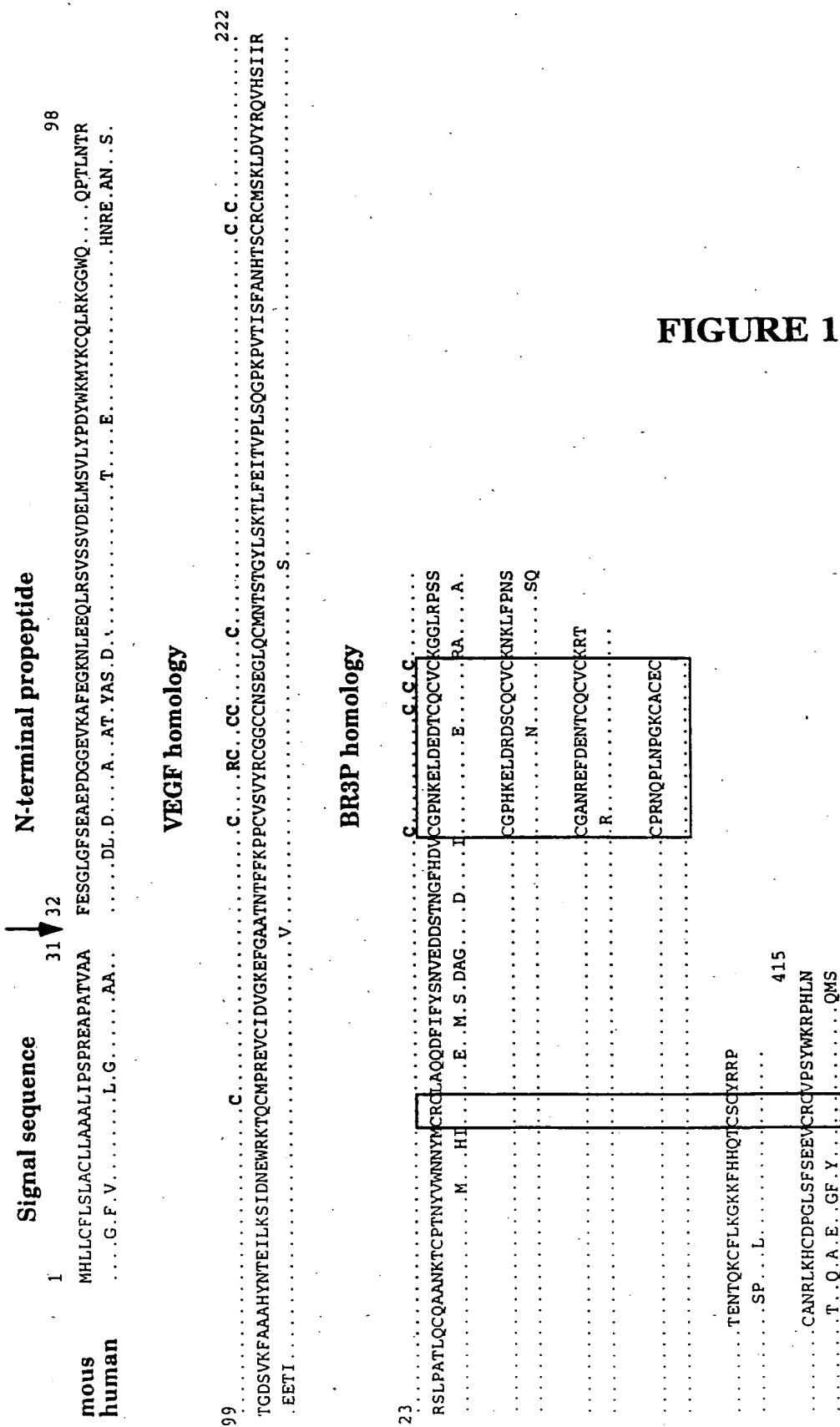


FIGURE 10

HUMAN		Donor site	Intron length	Acceptor site
Exon length				
.....	G...E...A...T(49).....A...Y...A...S.		
E1.....	GGC.GAG.GCC.ACG.gtaggtctgcgt...>10.kb..TTTCTTTGACAG.GCT.TAT.GCA.AGC			
.....	E...I...L...K(116).....S...I...D...N.		
E2.214.bp..	GAG.ATC.TTG.AAA.Agtaagtatggg...1.6.kb...atgacttgacagGT.ATT.GAT.AAT			
.....	L...S...K...T(180).....L...F...E...I.		
E3.191.bp..	CTC.AGC.AAG.ACG.gtgggtattgt.....9.kb.cccttctttag.TTA.TTT.GAA.ATT			
.....	T...L...P...Q(231).....C...Q...A...A.		
E4.152.bp..	ACA.CTA.CCA.CAGtgagtatgaattaaa.>10.kb..ttcttccaaagG.TGT.CAG.GCA.GCG			
.....	A...G...D... (266).....D...S...T...D.		
E5.107.bp..	GCT.GGA.GAT.Ggtagcagaatg.....301.bp...ctatttgtcttagAC.TCA.ACA.GAT			
.....	Q...T...C...S(378).....C...Y...R...R.		
E6.334.bp..	CAA.ACA.TGC.AGgtaagagatcc.....>10.kb..tgttctccttagC.TGT.TAC.AGA.CCG			
.....	Q...M...S(419)Stop.....			
E7.(501).bp..	CAA.ATG.AGC.TAA.GTATGTACTGTT...ATTGTATTAT			

FIGURE 11A

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MOUSE	Exon length	Donor site	Intron length	Acceptor site
E1	G...E...V...K(49)	GGC.GAG.GTC.AAG.gtagtgcaagg	>10.kb.attgtctttgacag	A...F...E...G.
E2	E...I...L...K(116)	GAG.ATC.CTG.AAA.Agtaagtag	4.kb...tgtgactcgacagGT	GCT.TTT.TGA.AGG
E3	L...S...K...T(180)	CTC.AGC.AAG.ACG.gtaggtat	9.kb...ttgtccctttag	S...I...D...N.
E4	T...L...P...Q(231)	ACA.TTA.CCA.CAGtgagtatg	10.kb.gtctccccaaaagG	ATT.GAT.AAT
E5	N...V...E...D(266)	AAT.GTT.GAA.GAT.Ggtaagtaaaa	350.bp	C...Q...A...A.
E6	Q...T...C...S(378)	CAA.ACA.TGC.AGgtaaggagtgt	6.kb.ttttcccctagt	TGT.TTT.GAA.ATT
E7	H...L...N(415) Stop	CAT.CTG.AAC.TAA.GATCATACC	polyA	TGT.TAC.AGA.AGA
E7	CAT.CTG.AAC.TAA.GATCATACC	ATTGTATTATAA	gctgtgaag	

FIGURE 11B

INTERNATIONAL SEARCH REPORT

Internat Application No

PCT/US 98/01973

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/52 C12N15/10 C07K16/24 C12Q1/68
 C12N15/62 G01N33/50 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N C12Q A01K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>JOUKOV V ET AL: "A NOVEL VASCULAR ENDOTHELIAL GROWTH FACTOR, VEGF-C, IS A LIGAND FOR THE FLT4 (VEGFR-3) AND KDR (VEGFR-2) RECEPTOR TYROSINE KINASES" EMBO JOURNAL, vol. 15, no. 2, 1996, pages 290-298, XP002022272 see the whole document</p> <p>---</p>	<p>1-5,7,8, 10,11, 30-34, 37-39,54</p>
X	<p>LEE, J., ET AL. : "vascular endothelial growth factor-related protein: a ligand and specific activator of the tyrosine kinase receptor flt4" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 93, March 1996, pages 1988-1992, XP002066360 see the whole document</p> <p>---</p>	<p>1-3,10, 11, 30-34, 37,38,54</p>

-/-

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

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- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- *Z* document member of the same patent family

Date of the actual completion of the international search

29 May 1998

Date of mailing of the international search report

03.07.98

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Holtorf, S

INTERNATIONAL SEARCH REPORT

Internal Application No

PCT/US 98/01973

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 39515 A (HUMAN GENOME SCIENCES INC ;ROSEN CRAIG A (US); HU JING SHAN (US);) 12 December 1996 pages 21,34; examples 2,3,4,5,6 ---	1-3,10, 11, 30-34, 36-38, 40,54
X	LEE,J., ETAL. : "VASCULAR ENDOTHELIAL GROWTH FACTOR RELATED PROTEIN (vrp): A LIGAND AND SPECIFIC ACITVATOR OF THE TYROSINE KINASE RECEPTOR Flt4" EMBL SEQUENCE DATA LIBRARY, 10 January 1996, HEIDELBERG, GERMANY, XP002066361 ACCESSION NO. U4142 ---	55,56
X	JOUKOV,V., ET AL. : "A NOVEL VASCULAR ENDOTHELIAL GROWTH FACTOR VEGF-C IS A LIGAND FOR THE FLT4 (VEGFR-3)AND KDR (VEGFR-2) RECEPTOR TYROSINE KINASES" EMBL SEQUENCE DATA LIBRARY, 1 February 1996, HEIDELBERG, GERMANY, XP002066362 ACCESSION NO. X94216 ---	55,56
A	COHEN T ET AL: "VEGF121, A VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ISOFORM LACKING HEPARIN BINDING ABILITY, REQUIRES CELL-SURFACE HEPARAN SULFATES FOR EFFICIENT BINDING TO THE VEGF RECEPTORS OF HUMAN MELANOMA CELLS" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 19, 12 May 1995, pages 11322-11326, XP002061896 see the whole document ---	1-57
P,X	WO 97 05250 A (UNIV HELSINKI LICENSING ;ALITALO KARI (FI); JOUKOV VLADIMIR (FI)) 13 February 1997 cited in the application pages 10,13-17, 27; examples 8,9,11,14,15,19,22,27,28,29; claims ---	1-11, 30-34, 36-40, 43,53-56
P,X	JOUKOV,V., ET AL. : "PROTEOLYTIC PROCESSING REGULATES RECEPTOR SPECIFICITY AND ACTIVITY OF VEGF-C" THE EMBO JOURNAL, vol. 16, no. 13, June 1997, pages 3898-3911, XP002066363 see the whole document ---	1-5, 7-11,14, 15,20, 21, 30-34,54
3 P,X	WO 97 09427 A (GENENTECH INC) 13 March 1997 pages 3,5,9,10,11,24,25,34; example 5,7 ---	1-3,10, 11, 30-38,54
3	---	-/--

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/01973

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 97 17442 A (IMMUNEX CORP) 15 May 1997 pages 3,7,8,17,19,21,24; examples ---	1-3,10, 11, 30-34, 36-38, 40,54
P,X	ACHEN, M.G., ET AL.: "VASCULAR ENDOTHELIAL GROWTH FACTOR D (VEGF-D) IS A LIGAND FOR THE TYROSINE KINASES VEGF RECEPTOR 2 (Flk1) AND vegf RECEPTOR 3 (Flt4)" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 95, January 1998, pages 548-553, XP002066364 abstract, pages 548, right column, page 549, 551; figures 1 + 4; ---	1-3, 30-34
P,X	JELTSCH, M., ET AL. : "HYPERPLASIA OF LYMPHATIC VESSELS IN VEGF-C TRANSGENIC MICE" SCIENCE, vol. 276, 30 May 1997, pages 1423-1425, XP002066365 see the whole document ---	1-3,10, 11, 30-32, 37-40, 53,54
T	JOUKOV, V., ET AL.: "A RECOMBINANT MUTANT VASCULAR ENDOTHELIAL GROWTH FACTOR-C THAT HAS LOST VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 BINDING , ACTIVATION, AND VASCULAR PERMEABILITY ACTIVITIES" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 12, 20 March 1998, pages 6599-6602, XP002066366 see the whole document -----	1-57

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/01973

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 37-47 and 49,50,52,53 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

The polypeptide analog of claim 13 was searched as referring to the analog mentioned in claim 12.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/01973

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9639515 A	12-12-1996	AU 6046796 A EP 0837934 A	24-12-1996 29-04-1998
WO 9705250 A	13-02-1997	AU 6616996 A EP 0842273 A	26-02-1997 20-05-1998
WO 9709427 A	13-03-1997	AU 7012896 A	27-03-1997
WO 9717442 A	15-05-1997	AU 1116297 A	29-05-1997